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Genetics of MuB1 and of a complement defect in inbred strains of mice

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1. INTRODUCTION

Macromolecules from individuals of the same species may show considerable differences. The existence of these differences, first recognized amongst antigens of erythrocytes and tissue cells, was later found amongst soluble proteins (reviewed by Cinader & Dubiski, 1963*a*), particularly the gamma globulins. Polymorphism in this latter class has been recognized by means of intraspecies antibodies and has been designated 'allotypy' (Oudin, 1956). Individuals of the same species may also differ in the presence or absence of a given macromolecule, and the present paper deals with an inherited variation of this latter type. The importance of both types of intraspecies variation lies not only in their usefulness as genetic markers but also in their possible effect on the inheritance of ability to make antibodies of certain specificities. This follows from the hypothesis that determinants to which individuals cannot make antibody have structures identical with those of autologous tolerance-inducing determinants (Cinader, 1960, 1961; Cinader & Dubiski, 1963*a*).

The discovery of a haemolytic-complement defect (Herzenberg *et al.*, 1963) and of an antigen (MuB1) deficiency (Cinader & Dubiski, 1963*b*, 1964; Erickson *et al.*, 1964) in certain strains of mice, has led to a study of the nature of the complement defect and of the antigen defect and to the realization that these two characteristics are associated. The state of antigen deficiency arises from complete lack of the antigen and not from reduced synthesis of it (Cinader & Dubiski, 1964). The complement defect is probably due to the absence of a factor isofunctional with the human C′5 component (Nilsson & Müller-Eberhard, 1965) and with the guinea pig 3′b or 3′d components (Linscott & Nishioka, 1963; Cinader, Dubiski & Wardlaw, 1964, 1965).

A sex-associated difference in the concentration of the antigen MuB1 and of the haemolytic complement titre (Cinader, Dubiski & Wardlaw, 1964, 1965; Terry, Borsos & Rapp, 1964) of animals possessing MuB1 has been observed. It is the purpose of this study to examine in detail the inheritance of the serum protein MuB1 and of the complement deficiency, and the nature of the relation between the MuB1 and the sex of offspring on the one hand, and of gamma globulin allotypes, on the other.

2. MATERIALS AND METHODS

(i) *Sources of mice*

Inbred strains of mice were obtained from Jackson Laboratories, except those not designated by /J which came from sources given in Table 1 of a previous article (Cinader, Dubiski & Wardlaw, 1964). Hybrid mice were obtained from Jackson Laboratories and backcrossed in our laboratory.

(ii) *Immunization of mice*

Mice were immunized by subcutaneous injection of an emulsion of MuB1-positive mouse serum in complete Freund's adjuvant.

(iii) *Preparation of heterologous antisera to MuB1*

Rabbits were injected subcutaneously with precipitates prepared from mixtures of MuB1-positive mouse sera and antisera to MuB1 of mouse origin. These precipitates were incorporated in complete Freund's adjuvant. Antibodies other than those directed against MuB1 were absorbed by adding to the resulting sera various quantities of MuB1-negative mouse sera or pseudoglobulin fractions obtained from MuB1-positive mouse sera. The mixtures were kept at 37°C. for 1 hour and precipitates were removed by centrifugation at +2°C.

(iv) *Double diffusion in agar*

Twenty-four ml. of molten agar (0·7% or 1·5%) were poured on to agar-coated glass plates (8·5 × 10 cm.). Holes cut in the gel with a punch were filled with reactants. When precipitin zones were observed with an immune serum, the specificity of the reaction was checked by a parallel test in which normal sera of the same strain as the immune sera were put into corresponding holes.

The quantity of MuB1 in a sample was estimated by serially diluting it and adding a portion of each dilution to peripheral holes around a central hole containing anti-MuB1 serum. The highest dilution giving a discernible zone was taken as the end point.

(v) *Assay of complement*

Volumes of sera equal to 0·1, 0·05, 0·02, and 0·01 ml. were delivered into pyrex tubes (10 × 75 mm.) held in an ice bath and made up to 0·1 ml. by addition of veronal buffer (Kabat & Mayer, 1961). To each mixture was then added 0·05 ml.

of a suspension of sheep erythrocytes (1·25% v/v) which had been sensitized immediately beforehand with a very large amount of antibody (haemolysin) as suggested by Rosenberg & Tachibana (1962). When used for the assay of guinea pig complement, 1·25% sensitized sheep cells were prepared by mixing equal volumes of 2·5% washed sheep erythrocytes and 1/8000 haemolytic serum. However, for measuring mouse complement, it was necessary to mix a 1/10 dilution of the haemolytic serum with 2·5% cells in order to obtain adequately sensitized 1·25% (v/v) cells. After adding the sensitized cells to the diluted mouse sera, the mixtures were incubated with periodic shaking for 1 hour at 37°C., whereupon 1·0 ml. of veronal buffer was added to each mixture and the tubes were centrifuged for 10 min. at 1500 r.p.m. and at 1°C. The degree of haemolysis was determined from the concentration of unhaemolysed cells, because of the complication of haemoglobin contributed by the mouse sera which frequently were haemolysed. To estimate unhaemolysed cells, the supernatants were poured off and the residues of unhaemolysed cells were deliberately haemolysed by adding 3·0 ml. distilled water. The optical densities of the tube contents were then measured at $\lambda = 410$ mμ. Control mixtures in which 0·1 ml. veronal buffer had been used in place of the dilution of mouse serum were processed in parallel and the optical density of these taken as representing 0% haemolysis during the incubation phase of the test. If the residual cells from the complement test mixtures gave optical densities close to this control value, the mouse sera were considered to lack detectable complement. Dilutions of mouse sera which resulted in an optical density of haemolysed residual cells equal to one-half of the control value were considered to contain one 50% haemolytic unit (HU_{50}).

3. RESULTS.

We shall first present data on the inheritance of MuB1 itself, then give genetic data linking the inheritance of MuB1 with that of complement and finally, present our finding on the relation between sex and gamma globulin allotypes, on the one hand, and MuB1 on the other.

(i) *Inheritance of the antigen MuB1*

F_1 hybrids from inbred strains of mice possess an antigen MuB1 whenever one or both parents carry this antigen, but not when both parents lack it (Cinader & Dubiski, 1964). MuB1 positive mice of strains C57BL/6J or C57L/J were crossed with MuB1-negative mice of strain A/J and the F_1 progeny were backcrossed to the MuB1-negative parents. The backcross progeny were bled at 5 to 7 weeks and tested by double diffusion in agar for the presence of MuB1 in their serum (Table 1). Approximately half these progeny were MuB1 positive, suggesting unifactorial inheritance of MuB1, determined by a single dominant gene. There was, however, some excess of MuB1-negative animals, perhaps due to a low sensitivity of the test for antigen. This excess was not statistically significant.

Table 1. *Distribution of MuB1 antigen in the offspring of the backcrosses*

Male parent	Female parent	Offspring MuB1+	Offspring MuB1−	Offspring Total	χ^2 and P(d.f.=1)
C57L/J♀ × A/J♂ (MuB1+)	A/J (MuB1−)	71 (73)	75 (73)	146	0·11 ($P>0·7$)
A/J (MuB1−)	C57L/J♀ × A/J♂ (MuB1+)	32 (39·5)	47 (39·5)	79	2·85 ($P>0·05$)
C57BL/6J♀ × A/J♂ (MuB1+)	A/J (MuB1−)	85 (94)	103 (94)	188	1·73 ($P>0·1$)
A/J (MuB1−)	C57BL/6J♀ × A/J♂ (MuB1+)	38	38	76	0·0
	Total	226 (244·5)	263 (244·5)	489	2·80 ($0·1>P>0·05$)

The expected numbers given in brackets are based on the assumption of unifactorial inheritance of MuB1.

(ii) *Link between the inheritance of complement deficiency and MuB1 deficiency*

Rosenberg & Tachibana (1962) demonstrated that some mice possess serum complement and others do not. Later Herzenberg *et al.* (1963) designated the factor responsible for the difference between these animals as *Hc*. A link between complement deficiency and the absence of antigen MuB1 (Cinader & Dubiski, 1963, 1964; Erickson *et al.*, 1964) became apparent in 1964 (Cinader, Dubiski & Wardlaw, 1964), and we shall present here the genetic evidence which showed this.

First we examined the status of twenty-four inbred strains of mice for the presence of MuB1 and of haemolytic complement. It will be seen from Table 2 that seventeen strains of mice possessed the antigen MuB1, and also the complete haemolytic complement system, and that the seven strains lacking MuB1 also lacked the haemolytic complement system. In view of this correlation, the link between MuB1 and a factor of complement was further explored by a genetic

Table 2. *The distribution of MuB1 and of a functional haemolytic complement system in some inbred stains of mice*

Strain	MuB1	Complement	Strain	MuB1	Complement
A/HeJ	−	−	DBA/2J	−	−
AKR/J	−	−	DBA/2DeJ	−	−
BALB/cJ	+	+	MA/J	+	+
BDP/J	+	+	P/J	+	+
BUB/Bn	+	+	PL/J	+	+
CBA/J	+	+	RF/J	−	−
CE/J	−	−	SL/R1	+	+
C57BL/Ha	+	+	SJL/J	+	+
C57BL/6J	+	+	SM/J	+	+
C57BR/cdJ	+	+	SWR/J	−	−
C57L/J	+	+	T6	+	+
C58/J	+	+	129/J	+	+

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Table 3. *Correlation between the presence of MuB1 and of haemolytic complement*

MuB1 and complement status of offspring	Number of offspring from mating: Hybrid 1♂ × A/J♀	A/J♂ × Hybrid 1♀	Hybrid 2♂ × A/J♀	A/J♂ × Hybrid 2♀	Total
MuB1 present					
Complement present	35	2	23	15	75
	(19·8)		(11·1)	(6·6)	
Complement absent	0	0	0	0	0
	(15·2)		(11·9)	(8·4)	
Subtotal	35	2	23	15	75
MuB1 absent					
Complement present	4	1	2	0	7
	(19·2)		(13·9)	(8·4)	
Complement absent	30	1	27	19	77
	(14·8)		(15·1)	(10·6)	
Subtotal	34	2	29	19	84
Total	69	4	52	34	159
χ^2	54·5	—	44·2	34·1	133·2
P (d.f. = 1)	< 0·005	—	< 0·005	< 0·005	< 0·005

The figures in brackets give the expected numbers calculated on the assumption that there is no correlation between MuB1 and haemolytic complement; in the bottom line the probability for this assumption is given.

Hybrid 1 = C57L/J♀ × A/J♂. Hybrid 2 = C57BL/6J♀ × A/J♂.

analysis of the inheritance of antigen MuB1 and haemolytic complement in the serum of offspring from backcrosses. Tests for antigen were carried out by double diffusion in agar and tests for haemolytic complement were made with sensitized sheep erythrocytes. The results in Table 3 show that there was good correlation between the presence of MuB1 and haemolytic complement and it may thus be concluded that the presence of MuB1 is necessary for the functioning of the haemolytic complement system: however, there was not complete agreement between the two tests. This may be attributable to relatively low concentrations of MuB1 and of complement in some of the backcrosses, possibly because of a single dosage effect which may result in a lower concentration of antigen in heterozygous than in homozygous animals. Also, the concentration of antigen and of complement may be such that the tests for their detection are being used at the limit of their sensitivity so that slight variations in concentrations may be the cause of failure in detection (Cinader, Dubiski & Wardlaw, 1964).

(iii) *The relation between MuB1 and gamma globulin allotype*

We turned our attention next to the question of whether there was a genetic link between MuB1 and the allotypes of gamma globulin. The possibility of there being such a link was suggested by the close functional association of complement and antibody molecules in immune processes. So far several allotypic gamma

Table 4. *The distribution of MuA2 and MuB1 in several inbred strains of mice*

MuA2-positive strains

MuB1 negative	MuB1 positive
NBL/N, NS/Fr, PHH	C57BL/6J, C57BL/10J, HR/De, SJL/J, SM/J, WH/Ht

MuA2-negative strains

MuB1 negative	MuB1 positive
AKR/J, AU, BUA/Wi, BUC/Wi BUE/Wi, CE/J, C3HHeJ, DBA/IJ DBA/2J, DB/Sp, DDK, DM/Ms, FAKI, GFF, IF/Bcr, I/FnLn, JU/Fa, KK, MaS/A, NC, NZB/B1, SMA/Ms, ST/J, YBR/HeWiHa	A/J, A/HeJ, BALB/cJ, BDP/J, BSVS/Sr, BRVR/Sr, BUB/Bn, BUB/Bn-C, BUB/Wi, CBA/J, C3Hf/BiOci, CHI/St, C57BR/cdJ, C57L/J, C58/J, F/St, FU, MA/J, MO/Ko, NZO/B1, P/J, PE/R1, PHL, PL/J, PS, RIII/J, RF/J, SEA/Gn-se, SEC/1Gn, SL/R1, STOLI/Lw, SWR/J, T6, 129/J, 2BC3H, 2C3H

globulin specificities have been identified in mice and the system to which these belong has been referred to by various designations, such as MuA (Dubiski & Cinader, 1963*a*, *b*), Gg (Wunderlich & Herzenberg, 1963), or Asa (Dray *et al.*, 1963). We have examined the serum of fifty-seven inbred strains of mice for the presence of the allotypic gamma globulin marker MuA2 and for MuB1 by double diffusion tests in agar. The two factors were not correlated (Table 4). We also examined the sera of offspring from a *double* backcross (C57BL/6J × A/J) × A/J with antisera specific for MuA2 and MuB1 and found (Table 5) that there was no genetic link between MuA2 and MuB1.

Table 5. *Test for correlation between the presence of antigen MuB1 and of the allotypic specificity MuA2*

		Offspring					
		MuB1+		MuB1−			χ^2 and
Male Parent	Female Parent	MuA2+	MuA2−	MuA2+	MuA2−	Total	*P*
Hybrid*	A/J	30	32	44	30	136	1·64
		(33·7)	(28·3)	(40·3)	(33·7)		$P > 0{\cdot}1$
A/J**	Hybrid	12	11	9	14	46	0·79
		(10·5)	(12·5)	(10·5)	(12·5)		$P > 0{\cdot}25$
	Total	42	43	53	44	182	0·51
		(44·4)	(40·6)	(50·6)	(46·4)		$P > 0{\cdot}25$

* C57BL/6J♀ × A/J♂ which is MuB1+, MuA2+.

** A/J is MuB1−, MuA2−.

Figures in brackets give the expected numbers calculated on the assumption of no correlation between the presence of MuB1 and MuA2. The right-hand column gives the probability that the differences between expected and observed values are not significant.

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(iv) *Sex-associated factors in the concentration of MuB1*

We turned our attention next to sex-associated differences in the concentration of MuB1 in strains possessing the factor. A lower concentration in serum from female than from male mice was consistently found in the thirteen strains and substrains examined (Table 6). The sera used in these tests were obtained from animals of different ages, but the age of males and females of the same strain was always the same. The wide variation in the antigen concentration of animals of the same sex, but of different strain and age, led us to examine, by single diffusion, the antigen concentration as a function of age. It was found that the content of MuB1 increases with age, especially in males, less in females. The difference in the concentration between the sexes could be found in sera from animals of all ages between 3 weeks and 6 months (Cinader, Dubiski & Wardlaw, 1964, 1965).

Table 6. *Relative quantities of MuB1 determined by double diffusion-dilution test*

Strain*	Dilution increment	Reciprocal of greatest dilution at which zone was observed	
		Male**	Female
BALB/cJ	1·2	4·3	3·0
BSVS/Sr	1·31	2·9, 2·9	1·7, 2·2
BUB/Wi	2	4	2
C58/J	1·31	2·9, 2·2	1·7, 1·7
CBA/J	1·2	6·2	5·2
DBA/1J	2	2, 2, 2, 2	1, 1
DBA/1JSn	1·31	3·7	2·2, 1·7
DBA/LiA	2	8, 4	2
DBA_f/Sp-D	2	4, 4, 4, 4	2, 2, 2, 2
MO/Ko	1·31	2·9	1·7
SJL/J	1·2	4	3·1
T6	2	4	2
WH/Ht	1·31	1·7	1·3

* Male and female individuals of the same strain were of the same age.
** Each figure represents result of the titration of the serum of one individual.

Having thus established that there is a sex-associated difference in the concentration of MuB1 we next investigated whether there was any correlation between the sex of parents or the sex of offspring and the presence or absence of MuB1 in the serum of offspring of backcross matings. It will be seen from Table 7 that correlation of MuB1 with the sex of the parents can be ruled out. Furthermore, there is no correlation between the sex of the hybrids and the presence or absence of MuB1 (Table 8).

We have thus found that whereas the presence or absence of MuB1 is not correlated with the sex of parents and offspring, the concentration of this antigen in

Table 7. *Test for correlation between the occurrence of antigen MuB1 and the sex of the parents*

Parental animals	Offspring MuB1+	Offspring MuB1−	Total	χ^2 P (d.f. = 1)
♂ MuB1+	156	178	334	
♀ MuB1−	(154·4)	(179·6)		0·098
♂ MuB1−	70	85	155	$P > 0·75$
♀ MuB1+	(71·6)	(83·4)		
Total	226	263	489	

Table 8. *Test for correlation between the occurrence of antigen MuB1 and the sex of the hybrids*

MuB1 and sex of offspring	Number of offspring from mating*: Hybrid 1♂ × A/J♀	A/J♂ × Hybrid 1♀	Hybrid 2♂ × A/J♀	A/J♂ × Hybrid 2♀	Total
MuB1+					
♂	42	13	43	17	115
	(37·9)	(15·4)	(40·7)	(21·0)	(114·6)
♀	29	19	42	21	111
	(33·1)	(16·6)	(44·3)	(17·0)	(111.4)
Subtotal	71	32	85	38	226
MuB1−					
♂	36	25	47	25	133
	(40·1)	(22·6)	(49·3)	(21·0)	(133·4)
♀	39	22	56	13	130
	(34·9)	(24·4)	(53·7)	(17·0)	(129·6)
Subtotal	75	47	103	38	263
Total	146	79	188	76	489
χ^2	1·85	1·21	0·45	3·41	0·005
P (d.f. = 1)	$P > 0·1$	$P > 0·25$	$P = 0·5$	$P > 0·05$	$P > 0·9$

* Both hybrids are MuB1+ and A/J is MuB1−. Hybrid 1 = C57L/J♀ × A/J♂. Hybrid 2 = C57BL/6♀ × A/J♂.

Figures in brackets give the expected numbers calculated on the assumption that there is no correlation between the incidence of MuB1 and the sex of the hybrids. The bottom line gives the probability that differences between observed and expected values are not significant.

the serum of male and female animals of the same age and strain is nevertheless different.

4. DISCUSSION

These experiments have shown that MuB1 deficiency and complement deficiency are inherited in a unifactorial manner and that MuB1 and the functional haemolytic complement system are inherited in a dominant or co-dominant manner. Thus a link between MuB1 and a factor of complement has been clearly established, but the genetic experiments do not prove the two factors to be identical. Proof of

identity could presumably be obtained by isolation of MuB1 and demonstrating that serum from complement deficient mice, mixed with highly purified MuB1, could serve as a source of haemolytic complement. Until such experiments have been carried out, the identity of MuB1 with a factor of complement should not be taken as settled. Whatever the final solution of this question, a fascinating problem arises out of the apparent well-being of complement deficient animals.

It is quite possible that some complement-mediated reactions do not require all the components needed for immune haemolysis. Support for this view emerges from the work of Stiffel *et al.* (1964), who showed that phagocytosis of *Salmonella typhimurium* occurred at a similar rate in mice containing the full complement system as in mice genetically lacking haemolytic complement activity. However, *in vivo* decomplementation of either strain with antigen-antibody aggregate markedly reduced the opsonizing effect of specific anti-*S. typhimurium* antibody This showed that at least certain components of complement—those removed by antigen-antibody aggregate—assisted phagocytosis *in vivo*.

Direct involvement of MuB1 in an *in vivo* immune process is indicated by studies of cutaneous anaphylaxis in MuB1-positive and -negative mice (Ben-Efraim & Cinader, 1964). Using certain antisera it was found that two phases of the cutaneous reaction were discernible in the positive animals while the parallel test in MuB1-negative mice showed only one phase. It is quite clear that the reaction which occurs in both types of animal, probably the Arthus reaction, cannot involve the *complete* haemolytic complement system. The question arises whether this reaction involves the first stages of the chain of complement reactions which would proceed quite normally up to the point at which the deficient complement factor becomes involved, or whether a system, completely independent of complement, exists.

Another aspect of MuB1 deficiency which has concerned us is its effect on the antibody response to MuB1. It has been previously suggested that the inheritance of ability to make antibodies of particular specificities may be predetermined by the autologous antigenic determinants of the immunized animal (Cinader, 1960, 1961; Cinader & Dubiski, 1963*a*). Of the many determinants of a *foreign* protein, only those different from the determinants of the tolerance-inducing autologous proteins are able to elicit an antibody response. Since the immunized animals as a consequence of their polymorphism differ in their protein composition, they may also differ in their responsiveness to the different determinants on a given protein. The inheritance of the antibody responsiveness to most antigens is multifactorial since it depends on the inheritance of many separate autologous proteins. On the other hand, responsiveness to an antigen, possessing only one determinant, is unifactorial since the inheritance of the antibody response depends on the inheritance of the ability to synthesize only one autologous macromolecule. This can be clearly seen in the case of the responsiveness to MuB1 where, in fact, animals possessing MuB1 do not synthesize anti-MuB1, while animals lacking MuB1 do make anti-MuB1. Hybrids between MuB1-positive and MuB1-negative animals possess MuB1 and therefore are not able to synthesize anti-MuB1. Amongst backcrosses of such hybrids, 50% possess MuB1 and 50% lack it. Thus 50% of

backcrosses, if injected with MuB1 would respond by antibody formation and 50% would not. One might, from observations of this kind, erroneously conclude that anti-MuB1 formation is under direct, single gene control and that it is recessive. However, in the case under discussion, it is quite evident that the inheritance of the potential to make anti-MuB1 is not directly under genetic control but is an indirect consequence of the genetic control of MuB1 synthesis. Thus the apparently recessive inheritance of anti-MuB1 potential is actually a consequence of the dominant inheritance of MuB1. Our findings with MuB1 thus support a previously expressed hypothesis regarding the inheritance of antibody specificity (Cinader, 1960).

A study by Levine *et al.* (1963) of the inherited responsiveness of guinea pigs to DNP-polylysine and to the copolymer of lysine and glutamine led to the conclusion that the responsiveness to these antigens was inherited in a unigenic, dominant manner. These findings are compatible with the previously mentioned hypothesis and might be attributable to the existence of an autologous lysine-containing determinant, inherited in a unigenic recessive manner.

SUMMARY

A mouse antigen, MuB1, has been shown by the study of backcrosses to be inherited in a unifactorial dominant manner. Its inheritance is not linked with gamma globulin allotypes, but has been shown to be linked with the presence of the haemolytic complement system. The concentration of MuB1 in the serum of several inbred strains of mice showed sex-associated differences; however, the presence of MuB1 did not depend on the sex of the parents or on the sex of the offspring. The inheritance of antibody responsiveness is discussed in terms of the inheritance of the antigen, MuB1.

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REFERENCES

BEN-EFRAIM, S. & CINADER, B. (1964). The role of complement in the passive cutaneous reaction of mice. *J. exp. Med.* **120,** 925–942.

CINADER, B. (1960). Specificity and inheritance of antibody response: a possible steering mechanism. *Nature, Lond.* **188,** 619–622.

CINADER, B. (1961). Quelques aspects de la tolérance immunologique à l'égard d'antigènes définis. *Annls. Inst. Pasteur,* **100,** 265–289.

CINADER, B. & DUBISKI, S. (1963*a*). The effect on immunogenicity of acquired immunological tolerance. *Colloques. int. Cent. natn. Rech. scient.* **116,** 255–305.

CINADER, B. & DUBISKI, S. (1963*b*). An alpha-globulin allotype in the mouse (MuB1). *Nature, Lond.* **200,** 781.

CINADER, B. & DUBISKI, S. (1964). Effect of autologous protein on the specificity of the antibody response: mouse and rabbit antibody to MuB1. *Nature, Lond.* **202,** 102–103.

CINADER, B., DUBISKI, S. & WARDLAW, A. C. (1965). Inheritance and properties of the antigen MuB1 and its relation to haemolytic complement. *Nature, Lond.* **205,** 97–98.

CINADER, B., DUBISKI, S. & WARDLAW, A. C. (1964). Distribution, inheritance, and properties of an antigen, MuB1, and its relation to hemolytic complement. *J. exp. Med.* **120,** 897–924.

DRAY, S., LIEBERMAN, R. & HOFFMAN, H. A. (1963). Two murine γ-globulin allotypic specificities identified by ascitic fluid isoprecipitins and determined by allelic genes. *Proc. Soc. exp. Biol. Med.* **113,** 509–513.

DUBISKI, S. & CINADER, B. (1963*a*). A new allotypic specificity in the mouse (MuA2). *Nature, Lond.* **197,** 705.

DUBISKI, S. & CINADER, B. (1963*b*). A new allotypic specificity in the mouse (MuA2). *Can. J. Biochem. Physiol.* **41,** 1311–1315.

ERICKSON, R. P., TACHIBANA, D. K., HERZENBERG, L. A., & ROSENBERG, L. T. (1964). A single gene controlling hemolytic complement and a serum antigen in the mouse. *J. Immun.* **92,** 611–615.

HERZENBERG, L. A., TACHIBANA, D. K., HERZENBERG, L. A. & ROSENBERG, L. T. (1963). A gene locus concerned with hemolytic complement in *Mus musculus. Genetics,* **48,** 711–715.

KABAT, E. A. (1961). Kabat and Mayer's *Experimental Immunochemistry,* 2nd ed., p. 241. Springfield, Illinois: Charles C. Thomas.

LEVINE, B. B., OJEDA, A. & BENACERRAF, B. (1963). Studies on artificial antigens. III. The genetic control of the immune response to hapten-poly-L-lysine conjugates in guinea pigs. *J. exp. Med.* **118,** 953–957.

LINSCOTT, W. D. & NISHIOKA, K. (1963). Components of guinea pig complement. II. Separation of serum fractions essential for immune hemolysis. *J. exp. Med.* **118,** 795–815.

NILSSON, U. & MÜLLER-EBERHARD, H. J. (1965). Immunologic relations between human B1F-globulin and mouse MuB1 (HC). *Fedn Proc. Fedn Am. Socs exp. Biol.* **24,** 620.

OUDIN, J. (1956). L'"Allotypie" de certains antigenes proteidiques du serum. *C. r. hebd. Séanc. Acad. Sci., Paris,* **242,** 2606–2608.

ROSENBERG, L. T. & TACHIBANA, D. K. (1962). Activity of mouse complement. *J. Immun.* **89**, 861–867.

STIFFEL, C., BIOZZI, G., MOUTON, D., BOUTHILLIER, Y. & DECREUSEFOND, C. (1964). Studies on phagocytosis of bacteria by the reticulo-endothelial system in a strain of mice lacking hemolytic complement. *J. Immun.* **93**, 246–249.

TERRY, W. D., BORSOS, T. & RAPP, H. J. (1964). Differences in serum complement activity among inbred strains of mice. *J. Immun.* **92**, 576–578.

WUNDERLICH, J. & HERZENBERG, L. A. (1963). Genetics of a gamma globulin isoantigen (allotype) in the mouse. *Proc. natn. Acad. Sci., U.S.A.* **49**, 592–598.