Identification and Genetic Control of Two Rabbit α_2-Macroglobulin Allotypes*

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ABSTRACT: Rabbits were immunized with macroglobulin preparations isolated from the serum of other rabbits by ultracentrifugation and gel filtration. This elicited precipitating isoantibodies which distinguished two antigenically different genetic variants, *i.e.*, allotypes of serum α_2-macroglobulin (α_2M). Identification of these allotypes as α_2M was based on the specific precipitation of a highly purified protein preparation which migrated electrophoretically as an α_2-globulin and had a sedimentation coefficient of approximately 18 S. That the inheritance of these two allotypes is controlled by a pair of allelic genes is based on the allotypes present in 235 progeny from the six possible mating combinations; *e.g.*, when both parents were heterozygous, the expected 1:2:1 distribution of progeny was observed (P = 0.95–0.9). This α_2M locus, designated *Mtz*, was shown not to be linked to the X chromosome nor to the light-chain or heavy-chain loci of the immunoglobulins. The use of these allotypes for the study of biosynthesis and structure of α_2M is discussed.

A new powerful tool for identification of genetic variants of serum proteins was introduced by Oudin in 1956 when he showed that proteins derived from one rabbit were immunogenic in a second rabbit, leading to the production of isoantibodies. Genetic variants of serum proteins identified by their antigenic properties with isoantibodies were designated as allotypes.

Rabbits immunized with whole serum from another rabbit produced isoprecipitins which identified serum protein allotypes among the α-, β-, and γ-globulins (Dray and Young, 1958). Several γ-globulin allotypic specificities have been characterized (Oudin, 1960; Dray *et al.*, 1963; Hamers *et al.*, 1966; Dubiski and Muller, 1967; Kelus and Gell, 1967) and shown to be controlled by two series of alleles at two unlinked genetic loci. In fact, the first suggestion of a multichain structure came from this genetic evidence. The allotypic specificities for the immunoglobulins serve as genetic markers for the protein and reflect differences in amino acid sequence (Small *et al.*, 1965, 1966; Reisfeld *et al.*, 1965). Consequently, the allotypes have been important for studying the structure and biosynthesis of the immunoglobulins (Dray and Nisonoff, 1963; Lark *et al.*, 1965; Harris *et al.*, 1963; Adler *et al.*, 1966).

We are now directing part of our research toward identification and characterization of rabbit serum protein allotypes other than the γ-globulins. It is anticipated that such studies will be useful to our understanding of the genetic control of protein synthesis. As the first antigen for study, we have chosen the macroglobulin fraction of serum with the idea that high molecular weight proteins would likely exhibit allotypic variation and be more antigenic than proteins of lower molecular weight.

This paper presents evidence for two allotypic specificities associated with the α_2-macroglobulin. Progeny data show that these two specificities are controlled by allelic genes at an autosomal locus which is not linked to the *a* (heavy chain) or *b* (light chain) locus of immunoglobulins.

Materials and Methods

Rabbits. Rabbits bred in our own laboratory were used in these experiments. The rabbits were progeny from crosses of closed colonies of Flemish giants and New Zealand whites, originally obtained from the National Institutes of Health.

Analytical Methods. Immunoelectrophoresis was performed in 1% Agarose in 0.05 M sodium barbital buffer (pH 8.5) (Grabar and Williams, 1955). The sedimentation velocity of samples was determined in a Spinco Model E ultracentrifuge using the An-D rotor with 12-mm double-sector cells. The experiments were performed in 0.85% NaCl at 52,000 rpm with the temperature regulated at 20°. Disc electrophoresis was performed in 7.5 or 3.5% polyacrylamide gel (pH 9.4) as described by Williams and Reisfeld (1964).

Isolation of Macroglobulin. Crude macroglobulin preparations were isolated from the sera of individual rabbits by first employing ultracentrifugation, primarily to remove low density lipoproteins, and then gel filtration to remove proteins of lower molecular weight. The density of serum was raised to 1.063 by the addition of 2 ml of KBr–NaCl solution (density 1.346) to 10 ml of serum (Havel *et al.*, 1955). The serum was then centrifuged in the no. 65K rotor at 40,000 rpm in the Spinco Model L2-65 ultracentrifuge for 24 hr at 4°. The macroglobulins were found in the pellet and lower 1–2 ml in each tube. The upper 10–11 ml containing the

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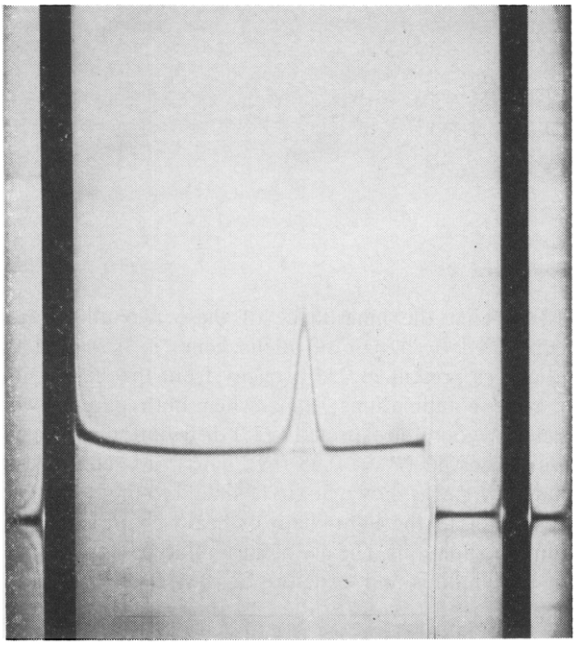

FIGURE 1: Ultracentrifuge pattern of purified α_2M in 0.85% NaCl. The centrifuge was run at 52,000 rpm and 20°. Photograph taken at 60° bar angle 16 min after attaining speed. Sedimentation is from right to left.

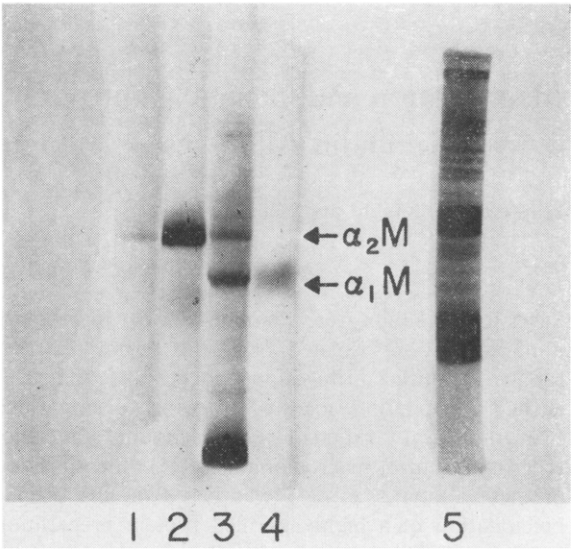

FIGURE 3: Disc electrophoresis in 3.5% polyacrylamide gel of purified preparations of α_2M and α_1M. Samples 1 and 2 are two different preparations of α_2M (0.5 and 6 mg/ml, respectively). Number 3 is whole rabbit serum and 4 is purified α_1M. Electrophoresis = 100 V for 1 hr. Sample 5 is whole rabbit serum in 7.5% polyacrylamide. Anode at bottom.

low density lipoproteins, as well as some of the other serum proteins, were removed, and the fraction containing the macroglobulins was placed on a Sephadex G-200 column equilibrated with 0.1 M sodium phosphate buffer (pH 7.4). The optical density of the eluate was determined at 280 mμ and three peaks were obtained. The macroglobulins were present in the first protein fraction eluted from the column.

This crude preparation of macroglobulins could be further purified on DEAE-cellulose. The protein in the first peak from Sephadex G-200 was dialyzed against 0.03 M acetate buffer (pH 5.1), and the resulting precipitate was removed. The supernatant was passed through DEAE-cellulose in 0.03 M acetate buffer (pH 5.1) (Got *et al.*, 1965). Most of the α_2-macroglobulin (α_2M) was not adsorbed by the DEAE-cellulose and α_2M was obtained in the eluate. Disc electrophoresis revealed that these preparations sometimes contained minor contaminants of other serum proteins.

In order to prepare a heterologous monospecific anti-α_2M antiserum, a preparation of α_2M completely free of contaminants was needed. Disc electrophoresis in 7.5% polyacrylamide gel was used to remove trace contaminants from the α_2M isolated by DEAE-cellulose. Approximately 75 μg of the α_2M was placed on each of 12 polyacrylamide gel columns. After electrophoresis, one tube was stained with Amido Black and the region corresponding to α_2M was cut from the remaining disks. The disk samples were pooled and crushed in a mortar and pestle.

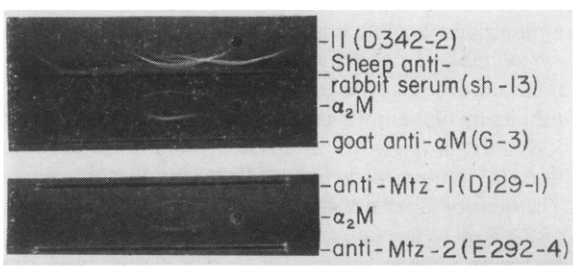

FIGURE 2: Immunoelectrophoresis in 1% Agarose of electrophoresed α_2M purified from a pool of rabbit sera with the two antiallotype sera and goat anti-α-macroglobulin. Electrophoresed rabbit serum (D342-2) with sheep antirabbit serum is shown as a reference. The anode is at the left.

α_1-Macroglobulin (α_1M) which was present in the crude preparations of macroglobulin was isolated by zone electrophoresis in a starch block with 0.05 M sodium barbital buffer (pH 8.6). Following electrophoresis, α_1M was eluted from the starch with the same buffer.

Antisera. Rabbits were injected with the crude macroglobulin preparation isolated by ultracentrifugation and gel filtration. The macroglobulins from Sephadex G-200 were concentrated by ultrafiltration to approximately 10 mg/ml and mixed with an equal volume of Freund's complete adjuvant (15% Arlacel A, 85% Bayol F, and *Mycobacterium butyricum*, 0.4 mg/ml). The rabbits were injected intramuscularly in the thigh twice a week for 3 weeks. A total of approximately 40 mg of protein was given to each animal. Another injection of approximately 5 mg was given in Freund's incomplete adjuvant 1 week after the last injection and the animals were bled 7 days later.

A goat was injected with α_2M isolated from disc electrophoresis. The crushed samples of acrylamide were mixed with Freund's complete adjuvant and a single injection was given in each thigh. Another intramuscular injection was given 3 weeks later in Freund's incomplete adjuvant. After 1 week, the goat was bled from the jugular vein and the serum was stored at −20°.

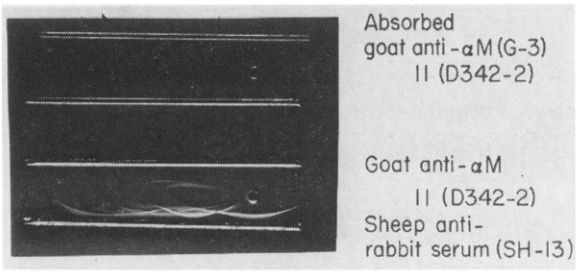

FIGURE 4: Immunoelectophoresis in 1% Agarose showing the precipitin reaction of electrophoresed rabbit serum (D342-2) and the goat anti-α-macroglobulin before and after absorption with α_1M. The anode is at the left.

Allotyping of Rabbits. Rabbits were bled from the marginal ear vein and the serum was separated and stored at −20°. The sera for typing of the progeny was obtained at 8–10 weeks of age. Double-diffusion experiments in agar gel were performed in 1.5% Noble Agar, 0.2 M borate, and 0.85% NaCl buffer (pH 7.9). A micro-Ouchterlony plate was used for routine typing of the rabbit sera using appropriate reference sera for controls as described previously (Dray *et al.*, 1963).

Results

Isolation of Macroglobulins. The macroglobulins isolated by gel filtration and DEAE-cellulose were shown to sediment as a single, symmetrical peak in the ultracentrifuge (Figure 1). A preliminary experiment using four different concentrations of α_2M yielded a straight-line plot of concentration *vs.* sedimentation coefficient which extrapolated to 18.6 S at zero concentration. This value is used in this paper only to establish that the proteins involved are macroglobulins. Further sedimentation studies are needed to determine the precise sedimentation coefficient but must await the availability of larger amounts of purified α_2M. Figures 2 and 3 show by the criteria of disc and immunoelectrophoresis that preparations of α_2M could be obtained which were highly purified. Immunoelectrophoresis of α_2M (5 mg/ml) revealed only one precipitin arc with sheep antirabbit serum even though the sheep antiserum had antibody to α_1M and γM, the most likely contaminants (Figure 2). Also, disc electrophoresis in 3.5% polyacrylamide gel revealed one major band and occasionally two to three minor contaminants (Figure 3, gels 1 and 2). However, some preparations of α_2M did contain small amounts of α_1-macroglobulin that could be detected by immunoelectrophoresis and disc electrophoresis.

The α_1M isolated by starch block electrophoresis gave a single peak in the ultracentrifuge with a sedimentation coefficient of approximately 19 S, uncorrected for concentration. By immunoelectrophoresis the α_1M revealed one precipitin arc with the sheep antiserum to rabbit serum. On disc electrophoresis, one band was found; however, since only approximately 10 μg was applied to the disk, minor contaminants may not have been detected (Figure 3).

Characterization of the Goat Antiserum. By immunoelectrophoresis, the goat antiserum prepared by

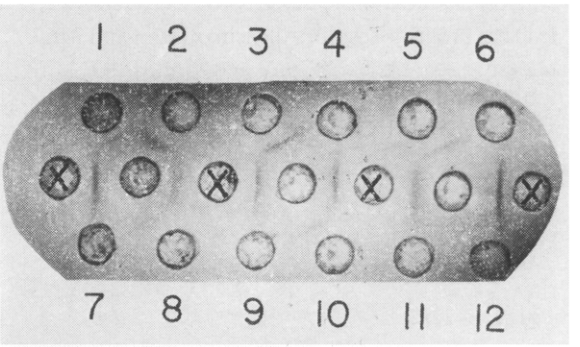

FIGURE 5: Double-diffusion experiment to demonstrate the method for typing rabbit sera to determine the allotypic specificities present. The sera to be typed were placed in wells 1–12. The antiserum was placed in the unmarked center wells and a reference serum was placed in the center wells labeled X.

immunization with an α_2-macroglobulin preparation reacted with whole rabbit serum to give two precipitin arcs (Figure 4). The major precipitin arc resulted from antibody to α_2M and the minor precipitin arc resulted from antibody to α_1M. That the goat antiserum was indeed specific for α_2M was confirmed by its reaction with an α_2M preparation which on immunoelectrophoresis against sheep antirabbit serum revealed a single precipitin arc in the α_2 region (Figure 2), and which had a sedimentation coefficient of 18.6 S (Figure 1). A monospecific goat anti-α_2M antiserum resulted from absorption of the goat antiserum with purified α_1M. In Figure 4, the serum was absorbed for immunoelectrophoresis by filling the goat antiserum trough with an excess of α_1M before the goat antiserum was added.

Identification of α_2M Allotype. By cross-immunization of rabbits with crude macroglobulin preparations, two antisera (D129-1 and D20-2) were obtained which were specific for different serum allotypes designated Mtz-1 and Mtz-2.[1] All normal rabbit sera examined reacted with one antiserum, the other antiserum, or both. Coalescence of the precipitin band of the unknown serum with the precipitin band of a reference serum was the criterion used to determine the presence of an allotypic specificity for each serum. As seen in Figure 5, the sera in wells 1, 2, 3, 5, 8, and 10 had the allotype designated Mtz-1, and the sera in wells 4, 6, 7, 9, 11, and 12 were negative for that allotype. Figure 6 is a photograph of an immunoelectrophoretic plate showing the precipitin reactions between the two antiallotype antisera (anti-Mtz-1 and anti-Mtz-2) and sera from rabbits of the appropriate allotype. Comparison of these precipitin arcs with those formed by the reference sheep antirabbit serum and by the goat anti-αM antiserum shows that the allotypes are found electrophoretically in the α_2 region. The goat anti-αM anti-

[1] The allotypes were designated Mtz-1 and Mtz-2 with the M relating to macroglobulin, t for the electrophoretic mobility (α-2), and z for the first locus to be identified. The 1 and 2 represent the alleles. This notation is only temporary until the biological significance of α_2M is known.

TABLE I: Progeny Test for Allelism of *Mtz*[1] and *Mtz*[2].

Parental Phenotype for α_2M[a]	No. of Litters	Total Progeny		Progeny Phenotypes for α_2M[a]			Probability
			b	z1z1	z1z2	z2z2	
z2z2 × z2z2	2	9	E	0	0	9	
			T	0	0	9	
z1z1 × z1z1	1	4	E	4	0	0	
			T	4	0	0	
z1z1 × z2z2	1	2	E	0	2	0	
			T	0	2	0	
z1z2 × z1z2	29	98	E	26	47	25	0.95–0.9
			T	24.5	49	24.5	
z1z2 × z1z1	20	69	E	38	31	0	0.5–0.3
			T	34.5	34.5	0	
z1z2 × z2z2	16	53	E	0	29	24	0.5–0.3
			T		26.5	26.5	

[a] The phenotype Mtz-1 and Mtz-2 are abbreviated to z1 and z2. [b] E = experimental; T = theoretical.

serum gave two precipitin bands, one for α_2M and one for α_1M. The appearance and position of the anti-allotype precipitin arcs were similar to the α_2M precipitin arc of the goat antisera.

α_2M, isolated from a pool of rabbit sera by gel filtration and DEAE-cellulose, reacted with both antiallotype antisera. On immunoelectrophoresis, the anti-allotype precipitin arcs were similar in position and appearance to each other and to the α_2M precipitin band formed with the goat anti-α macroglobulin antisera. Two additional antisera (D21-3 and E292-4) have been obtained which gave reactions identical with the two antisera described above.

Test for Allelism. Table I shows the phenotypes of 235 offspring from various matings. The probability values were obtained from the χ^2 test for the hypothesis that the allotypes Mtz-1 and Mtz-2 are controlled by allelic genes, *Mtz*[1] and *Mtz*[2]. None of the 98 progeny from matings in which both parents were heterozygous were negative for both allotypes. The probability values for all matings were greater than 0.3 and the experimental findings are, therefore, consistent with the hypothesis that *Mtz*[1] and *Mtz*[2] are alleles.

Test for No Association between the Mtz Locus and Sex. If the *Mtz* locus is not sex linked, 50% of the males from the matings of a homozygous animal with a heterozygous animal should be heterozygous for the two allotypic specificities. Of 66 male progeny from this type of mating, 34 (52.5%) were heterozygous. Table II presents the distribution of phenotypes with sex. The theoretical values were obtained by multiplying

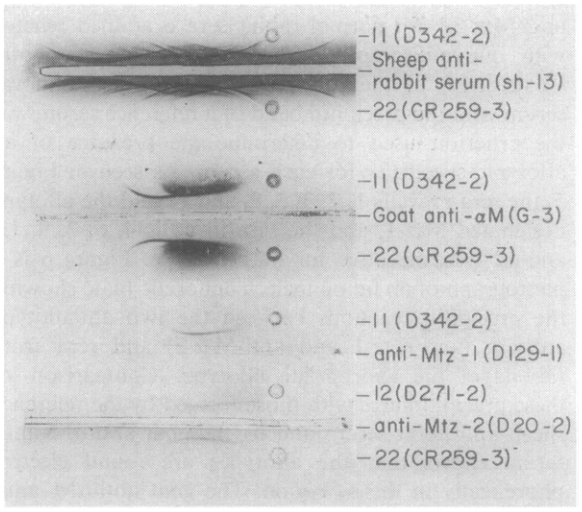

FIGURE 6: Immunoelectrophresis in 1% Agarose showing the precipitin reaction between electrophoresed rabbit serum and (1) sheep anti-whole rabbit serum, (2) goat anti-α-macroglobulin, and (3) antiallotype sera. The α_2M phenotype of each rabbit is given in front of the parentheses. The phenotypes Mtz-1 and Mtz-2 are abbreviated to 1 and 2. The anode is at the left.

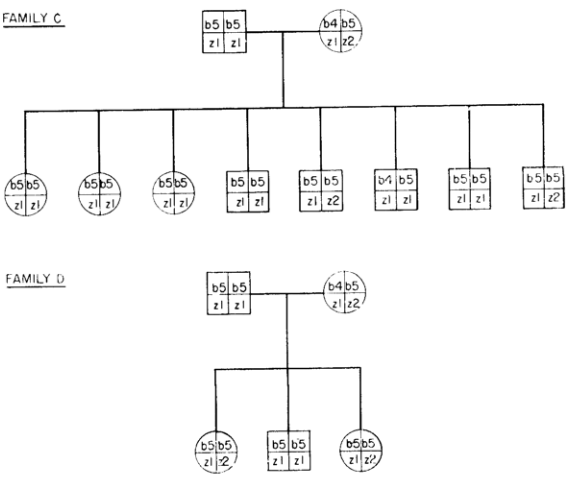

FIGURE 7: Pedigree of two rabbit families showing nonlinkage of the *b* (light chain) locus of immunoglobulins and the *Mtz* locus. The light-chain allotype is designated as b4 or b5 and the α_2M allotype is designated as z1 or z2.

TABLE II: Test for No Association between Sex and the *Mtz* Locus.

	No.		Phenotypes[a]			Probability
		b	z1z1	z1z2	z2z2	
Total population	295	E	81	145	69	
Males	152	E	41	75	36	0.98–0.95
		T	42	75	35	
Females	143	E	40	70	33	0.98–0.95
		T	39	70	34	

[a] The phenotypes Mtz-1 and Mtz-2 are abbreviated to z1 and z2. [b] E = experimental; T = theoretical.

the fraction of males or females in the total population by the number of each phenotype in the total population. The close agreement of experimental and theoretical findings indicates that the *Mtz* locus is independent of sex.

Test for No Association between the Mtz Locus of α-2 Macroglobulin and the Heavy-Chain (a) and Light-Chain (b) Loci of Immunoglobulins. Figures 7 and 8 show pedigrees of the *Mtz* and the immunoglobulin loci. If the *Mtz* locus and the *b* locus are closely linked, a double heterozygous parent b^4b^5 and z^1z^2 should transmit either the genes b^5,z^1 in half the gametes and in the other half, b^4,z^2 or the other combination of half b^5,z^2 and half b^4,z^1, but not both combinations. The two families represented in Figure 7 have progeny with genotypes b^5b^5,z^1z^1 and b^5b^5,z^1z^2, showing that the *Mtz* locus of α_2M and the *b* locus of immunoglobulins are not closely linked. In fact, there is independent assortment as indicated by the six progeny which received the b^5z^1 or b^4z^2 from the mother and the five progeny that received the b^5z^2 or b^4z^1.

TABLE III: Gene Frequency of the Two Alleles at the *Mtz* Locus in Various Rabbit Colonies from Roscoe B. Jackson Memorial Laboratory[a] and in Our Colony (FG/NZW).

Colony	No. Tested	Gene Frequency	
		Mtz^1	Mtz^2
ACep	26	100	0
AX	31	63	37
AC	10	95	5
III	30	77	23
III/X	14	68	32
OS	8	0	100
A	10	100	0
A_3	12	100	0
C	11	45	55
FG/NZW	295	52	48

[a] The gene frequencies represent the colonies as of 1958–1959 since the sera from the Jackson Laboratories were obtained at that time.

A similar example for the a locus is shown with the families in Figure 8. Both families show that the *Mtz* locus is not closely linked to the *a* locus of immunoglobulins. In family A, the a^1 gene of the father is transmitted with either z^1 or z^2. In Family B, the a^1 of the mother is transmitted with either the z^1 or z^2. Also, the a^3 of the father is transmitted with either the z^1 or z^2.

Population Studies. Table III shows the gene frequency of the *Mtz* locus for 153 rabbits from 9 colonies of the Roscoe B. Jackson Memorial Laboratory and 295 rabbits from our laboratory. For the calculation of gene frequency, it was assumed that only two alleles at the locus were present. The results show no indication of a third allele as no animals lack both Mtz-1 and Mtz-2, *i.e.*, sera from each animal react with anti-Mtz-1 and/or anti-Mtz-2.

Discussion

Two allotypes of rabbit α_2-macroglobulin, Mtz-1 and Mtz-2, have been identified by precipitin reactions with their specific isoantibodies. The isoantibodies were obtained by cross-immunization of rabbits with macroglobulin fractions isolated from serum of individual rabbits. The two allotypes are controlled by allelic genes; of 447 normal rabbit sera tested by the Ouchter-

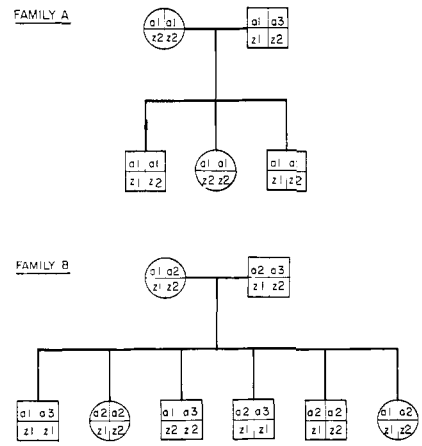

FIGURE 8: Pedigree of two rabbit families showing nonlinkage of the *a* (heavy chain) locus of immunoglobulins and the *Mtz* locus. The heavy chain allotype is designated a1, a2, or a3, and the α_2M allotype is designated z1 or z2.

lony technique, 171 were homozygous for Mtz-1, 82 were homozygous for Mtz-2, and 194 were heterozygous.

The two isoantibodies (anti-Mtz-1 and anti-Mtz-2) used to type the rabbit sera revealed only a single precipitin band on Ouchterlony plates and on immunoelectrophoresis. To minimize the possibility of cross-reactions, coalescence of the precipitin band with that formed by a reference serum was the criterion used to determine the presence of an allotypic specificity.

Although α_2M has not been shown to cross the maternal–fetal barriers (Brambell, 1963), the possibility was nevertheless considered in the interpretation of our data. That the α_2M present at 8–10 weeks of age was due to activity neonatal synthesis and not to maternal transfer is indicated by the following. The phenotype of rabbits at 5–6 months of age was the same as had been found at 8–10 weeks of age. The distribution of progeny from matings of heterozygous females with homozygous males gave no indication of an excess of heterozygous offspring as would occur if maternal transfer was a factor. For example, of 12 progeny obtained thus far from this type of mating, 7 were homozygous and 5 were heterozygous.

Although progeny tests indicated that the two allotypic specificities are controlled by allelic genes, the limited genetic data cannot eliminate the possibility of close linkage as occurs in the heavy-chain chromosome region of immunoglobulins (Oudin, 1960; Dray *et al.*, 1963, 1965; Kunkel and Natvig, 1968; Herzenberg, 1964). All 447 animals tested reacted with one or both of the antisera. Although no evidence of a third allele was found in any of the colonies from the Roscoe B. Jackson Memorial Laboratory or as a result of the matings in our laboratory, the possibility of additional alleles with a low gene frequency cannot be excluded.

Family studies showed that the *Mtz* locus is not closely linked to the *a* (heavy chain) locus or the *b* (light chain) locus of the immunoglobulins. This however, does not exclude the possibility of them being on the same chromosome.

The large number of heterozygous males in our experiments indicated that the rabbit *Mtz* locus is not sex linked. Matings of homozygous and heterozygous animals resulted in 51.5% of the males being heterozygous. This could only occur if the locus is not X linked. Also, the distribution of phenotypes and sex revealed that the phenotypes are independent of sex. A sex-linked locus for α_2M has, however, been identified in man by Berg and Bearn (1966), by using rabbit anti-whole human serum. Their antiserum reacted with the serum α_2-macroglobulin of some but not all individuals.

The rabbit allotypes described in our experiments were identified as α_2M by isolating a protein fraction by ultracentrifugation, gel filtration, and DEAE-cellulose, from pooled rabbit serum containing both allotypes, and analyzing this protein fraction by immunoelectrophoresis and ultracentrifugation. On immunoelectrophoresis, a single precipitin arc was observed in the α_2 region against sheep antiserum to rabbit serum and against each rabbit antiallotype antiserum. All three precipitin arcs had the same appearance and position. The protein preparation gave a single peak in the ultracentrifuge with a sedimentation coefficient of 18.6 S. This sedimentation value is provisional since it is based on only one experiment at four different concentrations. Difficulty in obtaining large quantities of α_2M, free of α_1M, have delayed extensive physiochemical characterization of the α_2M but isolation of such quantities is presently being undertaken.

The α_2M isolated from DEAE-cellulose was often contaminated by small amounts of α_1M. A contamination greater than approximately 1 μg could be seen on immunoelectrophoresis but was not easily detected by disc electrophoresis in 7.5% acrylamide, since under these conditions, the α_1M and α_2M migrate to similar positions. Thus, the α_2M cut from the discs, for injection of the goat, was deceptively pure, since it elicited two different antibodies (anti-α_1M and anti-α_2M). In later experiments, separation of α_2M and α_1M was obtained on disc electrophoresis by using 3.5% acrylamide.

Since the major contaminant of α_2M was α_1M, the potent goat antirabbit macroglobulin antiserum was an important reagent for detecting, on immunoelectrophoresis, small amounts of contamination by α_1M. The goat antimacroglobulin antiserum could also be absorbed with purified α_1M to obtain a monospecific goat antisera for rabbit α_2M and this will facilitate the quantitation of total rabbit α_2M.

The macroglobulins isolated by ultracentrifugation and gel filtration used for injection of rabbits contained at least three proteins (γM, α_1M, and α_2M). The contaminating proteins were of little concern since the rabbit would not make antibody to these homologous proteins unless the donor and recipient animals were allotypically different. Since, however, several different animals were used in these experiments, it is not surprising that an antibody to α_1M has been found and work is presently being undertaken to characterize this system.

Allotypes to α_2-macroglobulin have now been reported in at least three species. The human X-linked allotype (Berg and Bearn, 1966) was found with an absorbed heterologous antiserum. A Chimpanzee allotype of α_2M have been found by isoimmunization with α_2M (Lichter, 1968). A macroglobulin allotype in cattle has recently been reported (W. H. Stone, personal communication); however, it is not yet clear whether the protein is γM or αM. In all these reports, only one allele thus far has been identified and no evidence has yet been presented indicating whether they are structural genes. These data do suggest, however, that macroglobulin allotypes may commonly occur in several species.

The allotypic marker for α_2M described in this paper can be a useful tool for studying the synthesis, structure, and biological function of α_2M. Although little is known about the structure of α_2M, the molecule is probably composed of more than one polypeptide chain (Picard *et al.*, 1965). The relationship between these chains and the two allotypic specificities should be determined. The antigenic differences could result from differences

in the conformation of the molecules, the amino acid sequence, or prosthetic groups such as the carbohydrate moiety. Differences in the immunoglobulin allotypes have been attributed to differences in the amino acid sequence and it would seem likely that such would be the situation for the α_2M allotypes of the *Mtz* locus. However, the α_2M has a much larger per cent of carbohydrate and the assumption cannot be made at this time as to whether the *Mtz* locus is a structural gene for a polypeptide chain or a gene controlling the addition of a prosthetic group. Also, important aspects of the cellular biosynthesis of α_2M can be investigated. The allotypes provide an approach for studying the synthesis of a large molecule (approximately 900,000 mol wt) which otherwise would be very difficult. To begin, one could ascertain whether both allotypes are found in a single α_2M molecule and whether both allotypes are synthesized in the same cell. A comparison of these results with those obtained for the hemoglobin and immunoglobulin systems will be helpful in elucidating the biosynthetic mechanisms of serum proteins.

The function of α_2M remains unknown; however, the concentration of serum α_2M increases in chronic disease and infection. It has been shown that α_2M inhibits the proteolytic activity of trypsin and plasmin, and protects trypsin esterase activity from inhibition by soybean trypsin inhibitor (Mehl *et al.*, 1964; Ganrot, 1967; James *et al.*, 1966). Also, α-macroglobulins have been reported to increase the survival of X-irradiated mice (Hanna *et al.*, 1967).

Protein biosynthesis of the γ-globulin allotypes may be altered by exposing heterozygous b^4b^5 offspring to anti-b5 or anti-b4 antibody in fetal or neonatal life (Mage and Dray, 1965; Mage *et al.*, 1967). The presence of anti-b5 antibody resulted in the offspring having significantly lower levels of γ-globulin with the b5 allotype compared to normal animals. The same phenomenon occurred for the anti-b4 antibody and the suppression of the allotypes continued throughout the life of the animal. One question arising from these experiments is whether this suppression is unique for the immunoglobulins or may also occur in other systems, for example, the α_2M. Finally, as more serum protein allotypes are defined, the biological and genetic interrelationships between these proteins may become better defined.

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