SERUM β-GLOBULIN POLYMORPHISM IN MICE

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Summary

Data are presented to show that there are three serum β-globulin types in laboratory mice controlled by a pair of alleles. Each allele appears to give rise to three electrophoretically distinct zones in starch gel. Within the inbred strain A/AGS there was variation between mice in the intensity of staining of the three zones. Reciprocal mating data gave no evidence of an effect of β-globulin type on segregation ratios as has been reported for cattle.

I. Introduction

Following the initial recognition of serum β-globulin polymorphism in cattle independently by Ashton (1957, 1958*c*), Hickman and Smithies (1957), and Smithies and Hickman (1958), it was soon established that the same phenomenon occurs in other mammals. Thus Smithies (1957) showed that human β-globulins are polymorphic while Ashton (1958*d*, 1958*e*, 1960*a*) and Ashton and McDougall (1958) demonstrated β-globulin polymorphisms in the serum of a number of farm animals including sheep, horses, goats, and pigs. Recently McDougall (personal communication) has found the phenomenon in red deer.

Other examples of serum protein polymorphism have been found. The original demonstration of qualitative inherited differences between the serum proteins of individuals was with the haemoglobin-binding α-globulins, termed haptoglobins (Smithies 1955). Polymorphic differences have also been found in the "thread-proteins" of cattle (Ashton 1958*a*) and pigs (Ashton 1960*a*), in the Sα proteins of cattle, and in the α-globulins and pre-albumins of horses (Ashton 1958*b*, 1958*d*). However, while all mammals so far examined have shown β-globulin polymorphism, the other polymorphisms have been seen in only one or a few species.

Relationships between β-globulin type and fertility (Ashton 1960*c*) and β-globulin type and economic factors (Ashton 1960*b*) have been examined in dairy cattle. It was found that matings between homozygous cows and homozygous bulls were significantly more fertile than matings between heterozygous cows and heterozygous bulls, a finding confirmed by Ogden (personal communication). Also it was shown that β-globulin type affected milk yield, β^{DD} cows being on average superior to β^{AA} cows by about 50 gal. These observations prompted a search for similar effects in other species. For this reason the serum β-globulins of another ruminant, the sheep,

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are being investigated. It was also considered likely that the mechanisms underlying these β-globulin effects would be more easily identified in an animal more amenable to experimentation than the cow. Accordingly serum β-globulin polymorphism has been looked for, and found, in laboratory mice, guinea pigs, and rabbits. Laboratory mice have been studied in some detail, and the results are presented below.

The genetics of β-globulin polymorphism seems fairly simple and similar for each species. In each there appears to be a single locus controlling serum β-globulin type. So far, 5 alleles have been recognized in cattle (Ashton 1959*b*), 8 in the human (Giblett, Hickman, and Smithies 1959), 9 in sheep (Ashton and Ferguson, unpublished data), 2 in goats (Ashton and McDougall 1958), and 2 in pigs (Ashton 1960*a*). These results provided a baseline for the investigation of mouse β-globulin polymorphism.

II. Materials and Methods

(*a*) *Strains of Mice Examined*

Sera were examined from mice of the inbred strains C57BL/AGS, CBA/AGS, A/AGS, and 101/AGS. The number of animals tested from each strain was 27, 26, 27, and 4 respectively. In addition mice from three outbred stocks were examined: a randomly bred albino stock from the Department of Veterinary Physiology, University of Sydney, the Laboratory Animals Bureau's grey stock, and $+T$, $+t^3$, t^3t^3, and $+t^{12}$ mice derived from Tt^3 and Tt^{12} mutant lines maintained by Dr. S. Gluecksohn-Waelsch, New York. The number of mice examined from these three stocks were 26, 16, and 24 respectively. Three wild mice caught at Prospect were also tested.

Special mating groups were set up using mice of known serum β-globulin phenotype as indicated in Tables 1 and 2. Account was taken of the number of progeny born and the number weaned and, where possible, the phentoype of every mouse weaned was established. The mice were not tested until they had reached at least 5 weeks of age. Of those born 85% were weaned, and 78% were classified according to serum β-globulin type. Blood was obtained from the tail by the method described by Adams (1960), which enables samples of 0·5 ml to be taken rapidly; 20 mice could be bled in 30 min.

(*b*) *Starch-gel Electrophoresis*

Determination of the serum β-globulin phenotype was made by starch-gel electrophoresis using the horizontal technique developed by Smithies (1955) with minor modifications to the apparatus. The gels were prepared from hydrolysed starch (purchased from the Connaught Laboratories, University of Toronto, Canada) using phosphate buffer at pH 7·6 as described elsewhere (Ashton 1958*c*) for cattle serum proteins. Alternatively, we have used the discontinuous buffer system of Poulik (1957) as modified by Dr. K. A. Ferguson. With this system the electrolyte in the electrode compartments is a solution containing 1·2 g of lithium hydroxide and 11·8 g of boric acid per litre. The gel was prepared with a buffer made by adding 90 volumes of a solution containing 1·6 g citric acid and 6·2 g tris(hydroxymethyl)aminomethane per litre to 10 volumes of electrolyte. With this system and an applied voltage across the gel of about 10–12 V/cm the β-globulin zones were effectively separated in about 3 hr. The serum samples were inserted on rectangular pieces of filter paper

1 cm wide. To avoid irregularity in the final pattern the paper inserts were removed 15 min after the electrophoresis commenced. Several samples and a reference sample were run side-by-side on each gel. After electrophoresis the gels were slit lengthwise and the two exposed surfaces stained with nigrosine (Ashton 1958*c*). The β-globulin phenotype was assessed by comparison of the patterns given by the samples with that of the reference serum on the same gel.

The characterization of the zones separated from mouse serum by one-dimensional electrophoresis was aided by the two-dimensional electrophoresis technique of Smithies and Poulik (1956), first in agar in borate buffer (Ashton 1958*c*) and then in starch gel in phosphate buffer.

TABLE 1

SEGREGATION OF MOUSE SERUM β-GLOBULIN TYPES

Parental Phenotypes	No. of Offspring of Phenotype		
	β^{AA}	β^{AB}	β^{BB}
$\beta^{AA} \times \beta^{AA}$	*	—	—
$\beta^{AA} \times \beta^{AB}$	70	75	—
$\beta^{AA} \times \beta^{BB}$	—	91	—
$\beta^{BB} \times \beta^{BB}$	—	—	*
$\beta^{BB} \times \beta^{AB}$	—	167	172
$\beta^{AB} \times \beta^{AB}$	6	15	5

* Matings between homozygotes were not specifically examined, but numerous examinations from animals of several strains (inbred and outbred) revealed only one type within each strain.

III. RESULTS

(*a*) *Serum β-Globulin Types*

Plate 1, Figure 1, shows three β-globulin phenotypes which were found during this work. Phenotype β^{BB} occurred as the sole β-globulin type in the inbred strains C57BL, A, and 101, in three outbred stocks of different origin, and in the three wild mice tested, while phenotype β^{AA} was seen in only one strain (CBA), where it was the sole type. The third phenotype was produced by crossing animals of phenotypes β^{AA} and β^{BB}. The progeny were all of the third phenotype β^{AB}. These results, and the progeny totals from the various mating types shown in Table 1 make it clear that β-globulin polymorphism in the mice examined is controlled by two alleles which we have called β^{A} and β^{B}, so that genotypes β^{A}/β^{A}, β^{A}/β^{B}, and β^{B}/β^{B} are represented by phenotypes β^{AA}, β^{AB}, and β^{BB}. Further, each allele controls three protein zones as resolved by starch-gel electrophoresis: a fast-moving, rather faint zone not easily seen in all gels, an intermediate, more intensely staining zone, and a slow-moving, intensely staining zone. Although the point has not been checked the different degrees of staining probably represent corresponding variation of quantity of protein present in the three zones, rather than different binding capacities of the individual proteins for nigrosine.

Usually the relationship between the intensity of staining of constituent zones for any β-globulin allele within a given species remains reasonably constant. However, within the A/AGS inbred line we have seen two β^{BB} types (Plate 1, Figs. 2 and 3). Each gives three zones in starch gel, the mobilities of the three zones being identical for each

TABLE 2

SEGREGATION DATA FROM RECIPROCAL MATINGS

Parental Phenotypes		Mating Group No.	No. Litters	No. Born	No. Weaned	No. of Female Progeny		No. of Male Progeny	
♀	♂					Homozygous	Heterozygous	Homozygous	Heterozygous
β^{AA}	β^{AB}	3	2	15	14	3	4	1	6
		4	2	8	8	1	3	4	0
		5	4	18	17	4	1	1	4
		6	3	12	9	1	2	2	4
		21	2	17	13	5	3	3	2
		22	2	12	7	1	3	3	0
Total			15	82	68	15	16	14	16
β^{AA}	β^{AA}	1	5	30	30	7	6	6	9
		10	3	34	25	6	4	10	5
		11	4	29	23	5	6	3	5
		12	3	26	25	4	7	7	6
Total			15	119	103	22	23	26	25
β^{BB}	β^{AB}	7	11	100	88	23	23	25	14
		8	9	77	67	14	19	13	15
		9	10	99	85	22	21	24	17
Total			30	276	240	59	63	62	46
β^{AB}	β^{BB}	2	4	33	27	9	10	4	4
		13	3	29	27	10	4	7	5
		14	4	31	30	9	5	5	5
		15	4	43	34	4	10	3	15
Total			15	136	118	32	29	19	29
Totals			75	613	529	128	131	121	116

"subtype". The most common type has zones which, in order of decreasing mobility, stain faintly, moderately, and intensely. The less common type has corresponding zones staining more or less evenly. When crossed with β^{AA} mice the resulting heterozygotes each showed three zones of corresponding mobility and were indistinguishable subjectively.

(*b*) *Reciprocal Mating Data*

Asymmetrical segregation ratios were obtained from matings between cattle of certain β-globulin genotypes (Ashton 1959*a*), so that there was a deficiency of offspring unlike the mother. This phenomenon was sought in mice.

Disturbed segregation ratios are most readily detected when two progeny phenotypes are expected in equal numbers from a mating type. Mice were therefore mated in four ways: $\beta^{AA}♀ \times \beta^{AB}♂$, and its reciprocal $\beta^{AB}♀ \times \beta^{AA}♂$; $\beta^{BB}♀ \times \beta^{AB}♂$, and its reciprocal $\beta^{AB}♀ \times \beta^{BB}♂$. The numbers of progeny observed of each phenotype is shown in Table 2. In none of the groups did the observed numbers differ significantly from the expected 1:1 ratio when tested by χ^2, nor was there any evidence of a significant excess of offspring of one sex in the combined data.

IV. Discussion

In common with other mammals the β-globulins of mice are polymorphic. As with all other examples of mammalian β-globulin polymorphism each allele appears to give rise to a group of zones on starch gel. In mice there seem to be three distinct zones per allele, in cattle four (Ashton 1959*b*), in sheep and goats two (Ashton and McDougall 1958), in horses three (Ashton 1958*e*), and in pigs three (Ashton 1960*a*). Recently Harris, Pennington, and Robson (1960) have demonstrated that there are probably two zones on starch gel for each β-globulin (transferrin) allele in the human.

It is possible that the β-globulin zones produced in each species from one allele are polymers of differing molecular size of a basic polypeptide. These would be separated in starch gel by the sieve-like action of the gel (Smithies 1955). However, the charge on each polymer would be the same, and supporting media not showing the sieve effect would not be expected to resolve the polymers. It has been demonstrated previously (Ashton and McDougall 1958) that while the β-globulin types in cattle, sheep, and goat sera may be recognized by paper electrophoresis, each allele gives rise on paper to one zone only, which cannot be resolved further.

The serum β-globulins of mice are unusual in that the relative staining intensities of the zones produced by an allele may differ between individuals within the same strain. Three individuals, showing the unusual pattern, from the A/AGS inbred line, were bled on two occasions about 2 weeks apart and the pattern had remained constant. The variation in relative staining intensity (and presumably in quantity of protein) of the zones in mice suggests a mechanism controlling the relationship between the constituent zones, although there is no evidence to distinguish between a genetic or physiological mechanism. The significance of the phenomenon is not known. However, it has been observed that in two sublines of the inbred strain, recognized by rejection of homografts, two individuals from one line had one β-globulin subtype, and two from the other line the other.

The data show no evidence of the disturbed segregation ratios that have been found in cattle, i.e. there is no consistent excess of female offspring of the same phenotype as the mother. It has been established that fertility in cattle is influenced by parental β-globulin type (Ashton 1960*c*), but that the mechanism is probably indepen-

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Fig. 1.—Anodic side of starch gel showing the stained protein zones from three different mouse sera after electrophoresis. The β-globulin phenotypes are, from left to right, β^{AA}, β^{AB}, and β^{BB}.

Fig. 2.—As for Plate 1, Figure 1, showing, from left to right, the phenotypes β^{BB}, β^{AA}, and modified β^{BB}. The latter phenotype illustrates clearly that each β-globulin allele produces three zones in starch gel.

Fig. 3.—As for Plate 1, Figures 1 and 2, showing, from left to right, the phenotypes β^{BB} modified, β^{AB}, and β^{BB}.

dent of that causing aberrant segregation ratios. The fact that the β-globulin mating groups in mice do not give asymmetrical segregation ratios does not necessarily mean therefore that parental β-globulin type has no effect on fertility.

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