FRACTIONATION OF SERUM ALBUMIN¹ AND GENETIC CONTROL OF TWO ALBUMIN FRACTIONS IN PIGS²

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Received October 29, 1965

A number of loci controlling the synthesis of serum proteins in the pig have been identified (KRISTJANSSON 1960a, b, 1961, 1963; ASHTON 1960; IMLAH 1964). McINDOE (1962) has described genetically controlled plasma albumin differences in the chicken and QUINTEROS *et al.* (1964) have reported similar differences in turkeys. STORMONT and SUZUKI (1963) have demonstrated genetic control of albumin phenotypes in horses and ASHTON (1963) has reported on serum albumin polymorphism in cattle. The key to detecting heterogeneity of albumin appears to be the use of gel buffers at acid pH values as indicated by McINDOE (1962).

It is evident that extension of our knowledge of the diversity of proteins and their genetic control will continue to depend upon developing new analytical methods that allow more discrete separation of proteins with different physical or chemical configurations.

This report describes a starch gel electrophoresis procedure for fractionation of pig serum albumin and presents evidence concerning genetic control of two albumin fractions.

MATERIALS AND METHODS

Sera obtained from the Animal Research Institute Yorkshire and Landrace herds were subjected to starch gel electrophoresis in gels made with 114 g of hydrolysed starch (Connaught Medical Laboratories, Toronto, Lot 190-1) in 1000 ml of a .01 M Tris(hydroxymethyl)amino-methane-.004 M citric acid buffer (pH 6.3). This was sufficient material for four gels. The starch was suspended in 250 ml of gel buffer at room temperature and the remaining 750 ml heated to 100°C before addition to the suspension. Other conditions of the analytical procedure were as reported by KRISTJANSSON (1963) with the exceptions that 165 v was applied for 75 minutes after removing the insert papers before increasing the voltage to 350 v, and the borate boundary was allowed to migrate 10.0 cm before ending the run.

Each serum sample was classified for albumin phenotype with respect to two albumin fractions. All classifications were carried out independently by three persons experienced in interpreting starch gel electrophoretic patterns. Reference sera which contained the two albumin fractions under study were selected and all samples were compared with these sera.

RESULTS AND DISCUSSION

The resolution which was achieved is illustrated in Figure 1. Two dimensional electrophoretic analyses were conducted (KRISTJANSSON 1960b) involving the

¹ Albumin in this paper, is defined as the major protein fraction with high mobility as indicated by KRISTJANSSON (1963).

² Contribution No. 214, Animal Research Institute, Research Branch.

FIGURE 1.—Separation of pig serum albumin components showing phenotypes observed for Albumin₁A and Albumin₁B.

first dimension in gels used for routine typing of transferrins, haptoglobins and prealbumins (KRISTJANSSON 1963) with the second dimension in gels prepared as above, and the reciprocal of these dimensions. These analyses showed that all of the protein fractions indicated in Figure 1 migrate at the same rate as the albumin fraction in the gel system described by KRISTJANSSON (1963). Prior migration in one gel system did not affect the migration behaviour of these proteins in the other system, indicating that no permanent alteration of mobility was induced in either system.

Thirteen subfractions of albumin having different mobilities in the gels at pH 6.3 were observed. The albumin fractions designated as Alb₁A and Alb₁B (Figure 1), have been studied to determine the genetic mechanism controlling their inheritance. The subscript is used to distinguish these proteins from other albumin differences which appear to be under control of different loci.

As illustrated in Figure 1, some sera contained only Alb₁A (phenotypes AO and AA), some contained only Alb₁B (phenotypes BO and BB), some contained both Alb₁A and Alb₁B (phenotype AB) and others contained neither Alb₁A or Alb₁B (phenotype OO). AO phenotypes contained approximately half the amount of Alb₁A observed in AA phenotypes and BO phenotypes contained approximately half the amount of Alb₁B observed in BB phenotypes. Similarly, AB phenotypes contained about half the Alb₁A and Alb₁B observed in AA and BB phenotypes. Using several variations of the above starch gel electrophoresis technique, we did not detect any protein consistently present in OO phenotypes and absent in AA, AB and BB phenotypes.

All six phenotypes were observed in our Yorkshire herd but 72 Landrace were all of the OO phenotype.

Among sires and dams which were sampled at eight months of age, there was no difficulty in distinguishing between BB and BO phenotypes as BB sera contained much more Alb₁B than any BO sera. However, some difficulty was en-

countered in distinguishing between these phenotypes among progeny sampled at four months of age owing to a number of sera which appeared to contain Alb₁B in amounts that varied between the BB and BO phenotypes observed in sires and dams. Similar problems were encountered in attempting to distinguish between AO and AA phenotypes in some pigs sampled at four months of age. This was due to sera from some of the young pigs containing proteins with similar mobility to Alb₁A and Alb₁B.

Progeny sera containing Alb₁B and no Alb₁A were used in a trial designed to test repeatability of typing for BB and BO phenotypes. Since repeatability of classifying these progeny phenotypes was not perfect either between operators or between analyses within operator the segregation data in Table 1 do not differentiate between BO and BB progeny nor between AO and AA progeny.

The distribution of progeny phenotypes in Table 1 is consistent with the hypothesis that the phenotypes observed are determined by three alleles, *Alb₁^A*, *Alb₁^B* which controls the synthesis of Alb₁A, *Alb₁^B* which controls the synthesis of Alb₁B, and *Alb₁^O* which has no detectable product under our analytical conditions. (Our attention has been drawn to the remarkable similarity between this hypothesis and that presented recently by RANDERSON (1965) with respect to genetic control of erythrocyte esterase phenotypes in the deer mouse.)

The primary point of agreement of the data with the above hypothesis lies in the fact that only progeny phenotypes expected under the hypothesis have been observed in each mating class. The hypothesis that the differences observed are due to two unlinked loci one controlling Alb₁A and the other Alb₁B, was rejected

TABLE 1

Distribution of albumin phenotypes of progeny of various matings*

Matings Sire Dam	BB + BO	Progeny phenotypes AA + AO AB		OO	P of χ^2
BB × BO	22	..	..	..	..
AO	6	..	3	..	..
OO	21	..	..	..	..
BO × AB	16	7	11	..	..
BO	20	..	..	8	..
AO	5	3	2	2	..
OO	47	..	..	63	.13
AO × BB	14	..	8	..	..
AB	5	10	11	..	..
BO	22	19	12	9	.07
AO	..	39	..	12	..
OO	..	55	..	76	.07
OO × BB	4	..	..	..	..
AB	12	5	..	..	..
BO	91	..	..	63	<.03
AO	..	23	..	14	.15
OO	..	..	..	55	..

* Albumin, in this paper, is defined as the major protein fraction with high mobility as indicated by KRISTJANSSON (1963).

TABLE 2

Distribution of progeny phenotypes from matings of BO and OO parents

Matings Sire Dam	Progeny phenotypes	
	AO	OO
AO × OO	55	76
OO × AO	23	14
Total	78	90
Heterogeneity $\chi^2 = 4.7$ ($P < .04$)		

TABLE 3

Distribution of progeny phenotypes from matings of BO and OO parents

Matings Sire Dam	Progeny phenotypes	
	BO	OO
BO OO	47	63
OO BO	91	63
Total	138	126
Heterogeneity $\chi^2 = 6.8$ ($P < .01$)		

because no AB or OO progeny were observed in the OO × AB mating class ($P < .00002$).

In the right hand column of Table 1, the probability values are given for the fit of the data to the expected ratios for those mating classes which appeared to give the poorest fit. None of these classes exhibited a particularly good fit to the ratios expected, and, where OO sires were mated to BO dams, a significant deviation from the expected 1:1 ratio was observed.

Four of the five classes with the poorest fits to expectation represent two sets of reciprocal crosses, and are presented in Tables 2 and 3. In Table 2, the matings of AO sires to OO dams and OO sires to AO dams are shown. The heterogeneity χ^2 has a probability value of less than .04 indicating that these two mating classes are not likely drawn from the same population with respect to their progeny segregation. In both of these classes, there was an excess of progeny of the same phenotype as the dam.

As shown in Table 3, the same situation was observed with respect to the mating of BO sires to OO dams and OO sires to BO dams.

The heterogeneity χ^2 has a probability value of less than .01 and an excess of progeny of the same phenotype as the dam was observed in both mating classes. The data presented in Table 1 are generally consistent in exhibiting a small excess of progeny with the same phenotype as their dams. Further information on these aberrant segregations is being gathered in order to determine whether they are part of a process which can be responsible for the maintenance of a balanced polymorphism.

The author wishes to thank MR. J. DICKIE and MR. W. ZEITZ for their contributions to this study.

SUMMARY

A starch gel electrophoresis system has been developed which separates pig serum albumin into 13 discrete fractions. Six phenotypes involving two of these fractions (Alb_1A and Alb_1B) have been observed and are designated as Alb_1AA , AB , BB , AO , BO and OO .—Data are presented which support the hypothesis that these phenotypes are determined by three alleles; Alb_1^{A} which controls the synthesis of Alb_1A , Alb_1^{B} controlling the synthesis of Alb_1B , and Alb_1^{O} which has no detectable product under the analytical conditions described.—Two sets of reciprocal crosses $\text{AO} \times \text{OO}$, $\text{OO} \times \text{AO}$ and $\text{BO} \times \text{OO}$, $\text{OO} \times \text{BO}$ exhibit heterogeneity of segregation. In each case an excess of progeny with the same phenotype as the dam was observed.

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