SERUM ALBUMIN POLYMORPHISM IN CATTLE

G. C. ASHTON¹

*C.S.I.R.O. Division of Animal Genetics, Cattle Research Laboratory,
Rockhampton, Queensland, Australia*

Received July 17, 1964

FIVE cattle serum albumin phenotypes resolved by starch gel electrophoresis have been described briefly by ASHTON and LAMPKIN (1964). It was suggested that the variation represented serum albumin polymorphism controlled by a locus with three alleles. Further observations on albumin phenotypes have been made on British and Zebu beef and dairy cattle in Australia and are the subject of this report.

MATERIALS AND METHODS

Serum samples examined: Serum samples were obtained from bulls, dams and offspring from the crossbreeding experiment at the National Cattle Breeding Station, "Belmont", Rockhampton, Queensland. The cattle sampled were F_1 Brahman $\times$ Shorthorn, Brahman $\times$ Hereford, Africander $\times$ Shorthorn, Africander $\times$ Hereford, and Shorthorn $\times$ Hereford, and the F_2 progeny from crosses within the F_1 breed groups. Some purebred beef Shorthorns from another property were also examined.

Serum samples from dairy cattle of the Australian Illawarra Shorthorn, Jersey, Guernsey and Friesian breeds were examined, and also a number of samples from the descendants of Sahiwal and Red Sindhi cattle imported into Australia from Pakistan.

Starch gel electrophoresis: The original detection of serum albumin polymorphism in cattle was made with whole serum in a discontinuous Tris-citric system at pH 8.6 (ASHTON and LAMPKIN 1964). Typing was found to be easier if the serum was diluted with five volumes of water before electrophoresis. With this system each phenotype gave either one or two discrete, well defined zones (Figure 1). Further work with a variety of continuous and discontinuous buffer systems has shown that the resolution of the albumin phenotypes is increased by lowering the pH of the gel buffer (see RESULTS).

In the chosen procedure the electrolyte and gel buffer have the same composition and contain 0.84 g citric acid and 1.0 g Tris(hydroxymethyl)aminomethane per litre of solution. The pH of this solution approximates 5.6 and is not critical. The gels were prepared from hydrolysed starch (Connaught Laboratories, Toronto, Canada) at the concentration recommended by the manufacturers, by the procedure described by KRISTJANSSON (1963). The electrophoresis was performed in a horizontal plane in an apparatus described previously (ASHTON and LAMPKIN 1964). With an applied current of 2.7 ma per cm width of gel, the albumin zones moved approximately 5 cm in 2½ hours.

RESULTS

Phenotypes: Three phenotypes, Alb A, Alb AB, and Alb B were found in a herd of Sahiwal cattle in Kenya. Three phenotypes, apparently the same, have been found in the descendants of Sahiwal cattle imported into Australia. It has not been possible because of quarantine restrictions to compare Kenyan and

¹ Present address: Department of Genetics, University of Hawaii, Honolulu 14.

FIGURE 1.—Four gels each with four serum samples showing albumin phenotypes. From left to right, Gel (a) stored sera; Alb A, Alb AB, Alb B, Alb AB. Gel (b) Alb A, fresh serum; Alb A, fresh serum; Alb AB, stored serum; Alb A, fresh serum. Gel (c) Alb A, stored serum; Alb A, fresh serum; Alb B, stored serum; Alb AB, stored serum. Gel (d) stored sera; Alb A, Alb B, Alb B, Alb AB. The anodic end of the gel is at the top of the photograph.

Australian sera directly, but it seems very probable that the three phenotypes seen in Australian Sahiwals correspond with those seen in Kenyan Sahiwals. Accordingly the three phenotypes seen in Australian cattle have been called Alb A, Alb AB and Alb B also. The rare Alb AC and Alb BC phenotypes seen in Tanganyikan Shorthorned Zebu cattle have not yet been seen in Australia.

Careful distinction must be made between the appearance of the starch gel phenotypes obtained by the electrophoresis of fresh sera and their appearance after storage of the sera. With fresh sera at pH 8.2 the Alb A and Alb B phenotypes each show a single zone, while Alb AB shows both these zones. As the pH of the buffer system is lowered each of these zones splits into two, producing a moderately staining faster zone and an intensely staining slower zone. At pH 5.6 these zones are well resolved (Figures 1 and 2). This phenomenon is similar

FIGURE 2.—Diagram showing appearance of albumin phenotypes in cattle (a) Alb A from fresh serum, (b) Alb A from stored serum, (c) Alb B from fresh serum, (d) Alb B from stored serum, (e) Alb AB from fresh serum, (f) Alb AB from stored serum. Direction of migration towards anode shown by arrow.

to that described by McINDOE (1962) for serum albumin phenotypes in the domestic fowl.

With serum which has stood on the bench for a few days, or has been stored at -17°C for some months, the appearance of the phenotypes is altered. Alb A and Alb B then show three zones, namely a fast moderately staining zone and two slower intensely staining zones (Figures 1 and 2). Alb AB also shows three zones which stain moderately, intensely, and moderately in decreasing order of mobility.

It will be obvious that care is needed in the interpretation of albumin phenotypes from cattle serum. Alb A is unlikely to be confused with the other phenotypes, but it is difficult to distinguish Alb AB from fresh serum and Alb B from stored serum. Alb AB and Alb B from stored serum are readily distinguished by the differences in relative staining intensity of the three zones and the slight difference in mobility of the fastest zone in the two phenotypes.

Mating results: The distribution of offspring from 460 matings from the National Cattle Breeding Station is shown in Table 1. The type of offspring obtained from a given cross is consistent with the suggestion advanced previously (ASHTON and LAMPKIN 1964) that the three albumin phenotypes Alb A, Alb AB and Alb B represent a polymorphism controlled by two codominant autosomal alleles Alb^A and Alb^B . The genotype Alb^A/Alb^A is thus represented by phenotype Alb A. Alb^A/Alb^B by phenotype Alb AB, and Alb^B/Alb^B by phenotype Alb B.

Breed distribution of albumin types: Alb^B has not been found in dairy and beef cattle of the common European breeds. It was absent in 39 Guernsey cows from one herd, 282 Jersey cows from five herds, and 81 Australian Illawarra Short-horn cows from two herds. It was not seen in a survey of 128 bulls from artificial breeding centres covering the common dairy breeds, including Friesian. None of the F_1 , F_2 , or F_3 progeny of beef Shorthorn $\times$ Hereford matings at the National Cattle Breeding Station have shown Alb^B , and it was absent from 31 beef Short-horn steers. Further representative sampling will be necessary to ensure that Alb^B is exclusive to Zebu cattle, but present indications are that it is.

The Brahman crossbred and Africander crossbred cattle on "Belmont" have

TABLE 1

Distribution of progeny phenotypes (observed/expected) from various mating classes and corresponding probability values of χ^2 for the observed/expected deviations

Matings (Alb phenotypes)		Progeny phenotypes			P
Male	Female	A	AB	B	
A $\times$ AA		171/171			
A $\times$ AB		35/44.5	54/44.5		<0.05
AB $\times$ A		27/22.0	17/22.0		<0.30
AB $\times$ AB		44/37.25	78/74.5	27/37.25	<0.30
AB $\times$ B			1/1	1/1	
B $\times$ AB			2/1.5	1/1.5	
B $\times$ B				2/2	

been derived from a small number of imported Brahman and Africander bulls, and so no valid estimate of gene frequencies in the two parental breeds can be obtained. The same applies to the Sindhi and Sahiwal crossbreds available for examination from the F. D. McMaster Field Station. The high frequency of *Alb^B* in their progeny, however, shows that this allele was present in at least some of the bulls of each of the four Zebu breeds introduced into Australia.

DISCUSSION

Isolated cases of "double albumins" have been described in humans (EARLE, HUTT, SCHMID and GITTEN 1959) and a genetical basis has been ascribed to the phenomenon. Polymorphism, according to FORD's (1940) definition, of serum albumins was first described by MCINDOE (1962) in the serum of the domestic fowl. STORMONT and SUZUKI (1963) have found albumin polymorphism in the serum of the horse and ASHTON and LAMPKIN (1964) in the serum of cattle. A double albumin has been found in a species of chipmunk by JOHNSON and WICKS (1959), and in a species of *Amphibolurus* lizard by KIRK and KIRSCH (personal communication). Serum albumin variation has been noted by BECKMAN, CONTERIO and MAINARDI (1963) in certain bird species and their hybrids. It seems likely that albumin polymorphism will prove widespread, as further species and breeds are examined.

In each of the species examined for which mating data are available the genetic hypothesis suggested is autosomal multiple allelism. The types of offspring obtained for each type of mating in the data reported in Table 1 are consistent with such a general hypothesis. The distribution of offspring from the mating *Alb A* male $\times$ *Alb AB* female however departs significantly ($P < 0.05$) from that expected from such a hypothesis. It seems unlikely that the two-allele theory advanced to explain these results is incorrect, in view of the results from other species exhibiting albumin polymorphism. Further, the mechanism of multiple allelism is well authenticated in several other serum protein polymorphic systems. The significant deviation from normal expectation suggests that the segregation ratios are aberrant rather than that the two-allele hypothesis is incorrect. In the aberrant mating there is an excess of offspring of the same genotype as the mother. A similar but not significant excess is seen in the reciprocal mating *Alb AB* male $\times$ *Alb A* female. These observations if confirmed by further mating data would imply differential viability of the genotypes *in utero*, and would warrant further investigation.

The reason for the very low frequency or complete absence of *Alb^B* in British breeds of cattle in Australia, compared with its apparently relatively high frequency in African and Indian breeds, is not known. The retention of *Alb^B* in Zebu cattle implies a selective advantage of this allele at least in the habitat in which Zebus are normally found, but the nature of the advantage is speculative. If *Alb^B* is found not to occur in European cattle this allele will be a useful marker for assessing the percentage of Zebu in herds in which European $\times$ Zebu crossing is practiced.

The AC and BC phenotypes found in Tanganyika shorthorned Zebu cattle were not seen in the present survey, and no mating data are yet available for these types. In the absence of contrary information, it is assumed that these phenotypes represent the genotypes Alb^A/Alb^C and Alb^B/Alb^C , the postulated allele Alb^C producing an albumin complex migrating more slowly than that from Alb^B . It has not been possible to determine whether the albumin produced by the postulated Alb^C also splits into multiple zones at pH 5.6.

The structural difference between the albumins produced by Alb^A and Alb^B has not been determined. The ease with which bovine serum albumin can be isolated and purified should make it a suitable material for studies similar to those of INGRAM (1957) and others on human hemoglobins, although the formation of multiple zones in starch gel will need to be considered. The presence of two zones per allele in fresh serum is reminiscent of the products of transferrin alleles in many species (ASHTON and McDUGALL 1958; ASHTON and BRADEN 1961), and of double zones from post-albumin alleles in cattle (ASHTON 1963). The reason for this double or multiple zoning remains obscure but is apparently a general phenomenon of serum proteins and not unique to transferrins. The change in phenotype on storage is possibly due to enzymic action. It is superficially similar to the action of bacterial neuraminidase on serum transferrins and other sialoproteins. However, it differs from these reactions in that the new protein formed during storage is faster in mobility than the original in the case of both Alb A and Alb B phenotypes (Figure 1). In all species examined removal of sialic acid from transferrin phenotypes has resulted in a decreased net charge and a corresponding decrease in mobility. The storage changes affecting the albumin phenotypes may result in dissociation of albumin polymers rather than increase in net charge due to removal of substituents.

STORMONT and SUZUKI (1963) found that the variation in albumin phenotypes in horses was paralleled by variation in postalbumin phenotypes. This is not the case in cattle, where the albumin and postalbumin (ASHTON 1963) genes segregate independently, as also do the transferrins.

Thanks are due to Mr. J. F. KENNEDY for access to cattle at the National Cattle Breeding Station, "Belmont", Rockhampton, Queensland, and to Mr. R. W. HEWETSON of C.S.I.R.O., Division of Animal Genetics, Wollongbar, New South Wales, for supplying blood samples.

SUMMARY

The results of 460 matings in a beef cattle herd show that albumin polymorphism in cattle is controlled by two autosomal codominant alleles Alb^A and Alb^B . While Alb^B is frequent in Zebu beef and dairy cattle it is apparently absent in European cattle. The appearance of the phenotypes changes on storage and at acid pH.

LITERATURE CITED

- ASHTON, G. C., 1963 Polymorphism in the serum postalbumins of cattle. *Nature* **198**: 1117-1118.

- ASHTON, G. C., and A. W. H. BRADEN, 1961 Serum β -globulin polymorphism in mice. *Australian J. Biol. Sci.* **14**: 248-253.
- ASHTON, G. C., and G. H. LAMPKIN, 1964 Serum albumin and transferrin polymorphism in East African cattle. *Nature* (in press).
- ASHTON, G. C., and E. I. McDougall, 1958 Beta-globulin polymorphism in cattle, sheep, and goats. *Nature* **182**: 945-946.
- BECKMAN, L., F. CONTERIO, and D. MAINARDI, 1963 Serum protein variations in bird species and hybrids. *Rutgers Univ., Serol. Mus. Bull.* **29**: 5-8.
- EARLE, D. P., M. P. HUTT, K. SCHMID, and D. GITLIN, 1959 Observations on double albumin A genetically transmitted serum protein anomaly. *J. Clin. Invest.* **38**: 1412-1420.
- FORD, E. B., 1940 Polymorphism and taxonomy. pp. 493-513. *The New Systematics*. Edited by J. S. HUXLEY. Clarendon Press, Oxford.
- INGRAM, V. M., 1957 Gene mutations in human haemoglobin: The chemical difference between normal and sickle cell haemoglobin. *Nature* **180**: 326.
- JOHNSON, M. L., and M. J. WICKS, 1959 Serum protein electrophoresis in mammals—taxonomic implications. *Syst. Zool.* **8**: 88-95.
- KRISTJANSSON, F. K., 1963 Genetic control of two prealbumins in pigs. *Genetics* **48**: 1059-1063.
- McINDOE, W. M., 1962 Occurrence of two plasma albumins in the domestic fowl. *Nature* **195**: 353-354.
- STORMONT, C., and Y. SUZUKI, 1963 Genetic control of albumin phenotypes in horses. *Proc. Soc. Exptl. Biol. Med.* **114**: 673-675.