SERUM TRANSFERRIN *D* ALLELES IN AUSTRALIAN CATTLE

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Summary

Transferrin phenotypes supporting the occurrence of two Tf^D alleles in cattle are illustrated. Segregation data and gene frequencies for five breeds for the two alleles Tf^{D1} and Tf^{D2} are presented.

I. INTRODUCTION

Serum transferrin polymorphism in *Bos taurus* cattle was described independently by Ashton (1957, 1958) and by Hickman and Smithies (1957) and Smithies and Hickman (1958). Each laboratory presented evidence for control by three co-dominant autosomal alleles which are now known as Tf^A, Tf^D, and Tf^E. Subsequently Ashton (1959) reported two further alleles, Tf^B and Tf^F, found so far only in *B. indicus* cattle, and Ashton and Lampkin (1964) reported another allele, Tf^G, found so far only in three related Boran cattle in East Africa. Gahne (1961) has described a phenotype, found in Icelandic cattle, which may represent the heterozygote of Tf^A and yet another allele similar in mobility to Tf^F.

Each of these transferrin alleles produces four zones in starch gel (Ashton and McDougall 1958), although in routine analysis the fastest zone is rather faint and is not always seen. This characteristic of three major zones per allele permits prediction of the appearance of the whole range of possible phenotypes when a "new" allele is discovered (Ashton 1959).

In 1962 Kristjansson reported that cattle Tf^D is in fact two alleles, each of which produces zones of very similar mobility in starch gel. A similar situation has arisen with sheep transferrins (Ashton and Ferguson 1963). The resolution of zones of very similar mobility has been accomplished by development of discontinuous buffer systems based on the original description of such systems by Poulik (1959).

The purpose of this paper is to illustrate the relationship of the two *D* alleles to the other transferrin alleles in *B. taurus* and *B. indicus* cattle and to present data on their distribution in some Australian breeds. The two *D* alleles have been coded Tf^{D1} and Tf^{D2}, Tf^{D1} producing zones of slightly faster mobility than Tf^{D2}. Samples were exchanged with Dr. F. K. Kristjansson in Ottawa, and it was found that the two alleles coded Tf^{D1} and Tf^{D2} in Rockhampton corresponded with the two alleles coded Tf^D and Tf^{D1} by Kristjansson (1962). It has been agreed to code these alleles Tf^{D1} and Tf^{D2} (Ashton and Kristjansson 1965).

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II. Methods

(a) *Starch-gel Electrophoresis*

The system used has been described previously (Ashton and Braden 1961; Ashton and Ferguson 1963; Ashton and Lampkin 1965) and has been in use since 1959. It is based on the discontinuous buffer system of Poulik (1959), and was developed by Dr. K. A. Ferguson for resolution of pituitary proteins in starch gel. It differs from Poulik's system firstly in the use of lithium hydroxide instead of sodium hydroxide, which lowers conductivity and hence lessens heat production without sacrificing ionic strength, and secondly in the admixture of a proportion of electrolyte with the gel buffer. This has the effect of slowing the transferrin zones relative to albumin, and gives excellent resolution of the numerous zones between the trailing edge of the albumin zone and the leading transferrin zone.

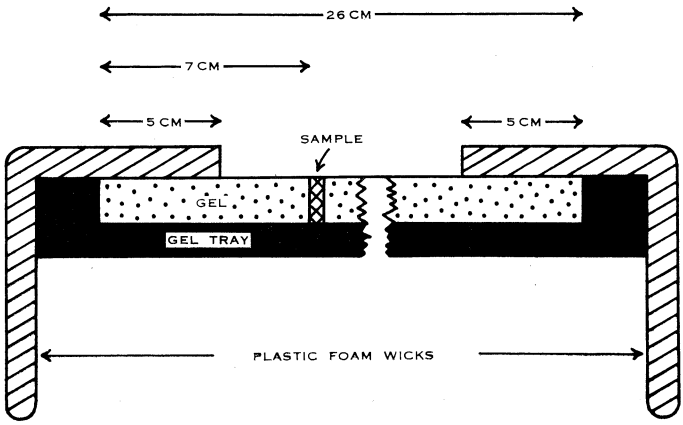

Fig. 1.—Showing gel dimensions and position of insertion of sample (on Whatman 17 filter paper) in relation to wicks.

The electrolyte consists of 0·75 g lithium hydroxide and 11·8 g boric acid per litre of solution, giving a pH of 7·8. The gel buffer consists of 450 ml of a solution containing 1·6 g citric acid and 4·8 g tris(hydroxymethyl)aminomethane per litre (pH 8·0) and 50 ml of electrolyte. Gels are prepared from Connaught hydrolysed starch (Connaught Laboratories, Toronto, Canada) at the concentration recommended by the manufacturer, by the method described by Kristjansson (1963). The gels are cast in moulds 26 cm long divided into units 4 cm wide and 0·3 cm deep. The gels are used within 2 hr of pouring.

The dimensions of the gel are fairly critical especially in regard to the point of sample insertion. Figure 1 shows the relationship of connecting wicks, sample insertion, and mould length. Samples are inserted on pieces of Whatman 17 filter paper, and removed after 20 min electrophoresis. During electrophoresis the gels are covered with thin polyvinyl film to prevent evaporation.

The applied voltage should be sufficiently high to effect rapid migration, but not so high that the gels get too hot. Gel temperatures of 40–45°C do not hinder resolution, and in fact seem to enhance it. With an applied voltage of 400 V across the apparatus, and an initial current of 4 mA per centimetre width of gel, electrophoresis is complete in 2–2½ hr. The voltage drop between the inside edges of the wicks is 180–190 V. Electrophoresis is terminated when the brown zone of discontinuity just reaches the inside edge of the anode wick.

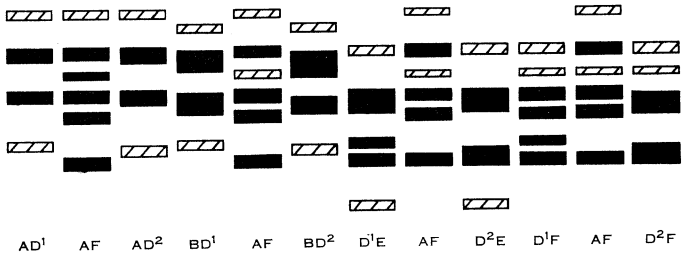

Fig. 2.—Showing pairs of *D* phenotypes referred to transferrin *AF*. The faint fastest zone produced by each allele is not shown. The cross-hatched areas indicate zones less intensely stained than the solid areas. See also Plate 1.

The undersurface of the gel is stained, either in 0·05% nigrosine or 0·1% naphthalene black in methanol–water–acetic acid (50 : 50 : 10 by vol.) and the gel is not sliced.

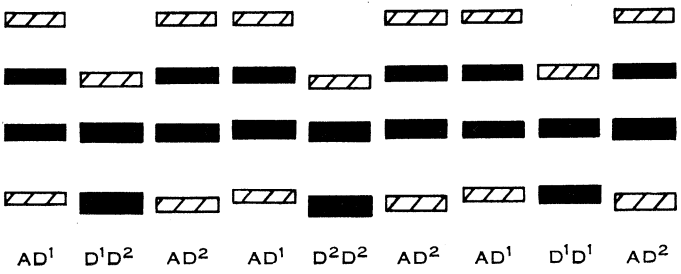

Fig. 3.—Showing *D* phenotypes referred to transferrin phenotypes D^1D^1, D^1D^2, and D^2D^2. The faint fastest zone produced by each allele is not shown. The cross-hatched areas indicate zones less intensely stained than the solid areas. See also Plate 1.

III. Results

(*a*) *Identification of Phenotypes*

Plate 1 shows some of the 21 phenotypes possible with the six alleles Tf^A, Tf^B, Tf^{D1}, Tf^{D2}, Tf^E, and Tf^F.

Distinction between Tf^{D^1} and Tf^{D^2} is most easily made in the heterozygous transferrin pairs AD^1 and AD^2, BD^1 and BD^2, D^1E and D^2E, D^1F and D^2F (Plate 1). Figure 2 illustrates the differences diagrammatically, in each case against transferrin AF which has been found most useful as a general reference for all cattle transferrin phenotypes except as noted below.

TABLE 1

RESULTS OF 71 MATINGS INVOLVING D PHENOTYPES

Parents	Phenotypes of Offspring	Parents	Phenotypes of Offspring
$AA \times D^1D^2$	1 AD^2	$EE \times D^1D^2$	1 D^2E
$AA \times D^2D^2$	3 AD^2	$EE \times D^2D^2$	3 D^2E
$AA \times AD^1$	4 AD^1	$EE \times AD^1$	1 AE
$AA \times AD^2$	13 AA; 11 AD^2	$EE \times AD^2$	4 AE; 5 D^2E
$AA \times D^2E$	1 AE	$EE \times D^2E$	1 D^2E
$AF \times D^1D^2$	1 AD^2; 1 D^2F	$FF \times D^1D^2$	4 D^2F
$AF \times D^2D^2$	1 AD^2	$FF \times AD^1$	1 AF
$AF \times AD^1$	2 AA; 1 AF; 1 D^1F	$FF \times AD^2$	6 AF; 5 D^2F
$AF \times AD^2$	3 AA; 2 AF; 3 D^2F; 3 AD^2		

The most difficult phenotypes to distinguish are D^1D^1, D^1D^2, and D^2D^2. Logically a D^1D^2 reference sample could be used, but in practice either AD^1 or AD^2 has proved more reliable (see Plate 1 and Fig. 3).

TABLE 2

GENE FREQUENCIES CALCULATED FROM PHENOTYPE DISTRIBUTIONS IN AUSTRALIAN COW AND BULL POPULATIONS

Breed	Source	No. of Animals	Gene Frequencies					
			Tf^A	Tf^B	Tf^{D^1}	Tf^{D^2}	Tf^F	Tf^E
Jersey	Cows in six herds	578	0·722	—	0·049	0·229	—	—
Jersey	Bulls at artificial breeding centres	51	0·598	—	0·147	0·255	—	—
Guernsey	Cows in one herd	47	0·500	—	0·330	0·170	—	—
Australian Illawarra Shorthorn	Cows in one herd	70	0·343	—	0·143	0·343	—	0·171
Friesian	Bulls at artificial breeding centres	30	0·483	—	0·100	0·367	—	0·050
Droughtmaster	Cows in one herd	293	0·266	0·072	0·041	0·319	0·111	0·191

(*b*) *Segregation of* Tf^{D^1} *and* Tf^{D^2}

Table 1 shows mating data supporting the conclusion that Tf^{D^1} and Tf^{D^2} are allelic to the other transferrin alleles. Because of the relatively low frequency of Tf^{D^1} in Australian cattle so far examined the critical matings $D^1D^2 \times D^1D^2$ and $D^1D^1 \times D^1D^1$

have not yet been encountered. No examples of a mating involving only the Tf^{D1} allele (in combination with other non-*D* alleles) giving a Tf^{D2} offspring have occurred. Similarly no matings involving the Tf^{D2} allele only have given progeny with Tf^{D1}.

(*c*) *Breed Distribution of* Tf^{D1} *and* Tf^{D2}

Table 2 shows the distribution of Tf^{D1} and Tf^{D2} and the calculated gene frequencies for Jersey, Guernsey, Australian Illawarra Shorthorn, and Droughtmaster breeds in Australia. These represent a limited number of herds and must be considered as preliminary estimates of breed gene frequencies. However, it would appear that Tf^{D2} is more frequent than Tf^{D1}, except in Guernsey cattle.

IV. Discussion

Recognition of "new" alleles presents several problems of which nomenclature is not the least. If the new allele produces zones clearly distinguishable from those of existing alleles, as in the case of Tf^{B}, Tf^{F}, and Tf^{G}, the practice of assigning the next available letter has some merit. However, it is essential that the same letter should not be allotted to different newly observed alleles. The establishment of a subcommittee to consider nomenclature of serum protein polymorphisms in farm livestock (cf. 1st Report of FAO Panel of Blood Group Scientists, AN 1963/7, Rome) should be useful in avoiding this pitfall. The problem of coding alleles which are recognized by improvement in technique, as in the case of the transferrin *D* alleles, is more complex. Coding each of the "new" alleles by addition of superscript numbers is unambiguous. It has the advantage of showing relationship to the previous coding, while emphasizing that the "new" alleles do not correspond with the old.

Recognition of two *D* alleles means that data relating transferrin type to milk yield (Ashton 1960; Ashton, Fallon, and Sutherland 1964) and transferrin type to fertility (Ashton and Fallon 1962) need reassessment. Preliminary results show that in the case of fertility at least there is no significant difference between D^1D^1, D^2D^2, and D^1D^2 bulls. The ranking of D^1D^1, D^2D^2, and D^1D^2 cows with regard to milk yield is being investigated.

V. Acknowledgments

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VI. References

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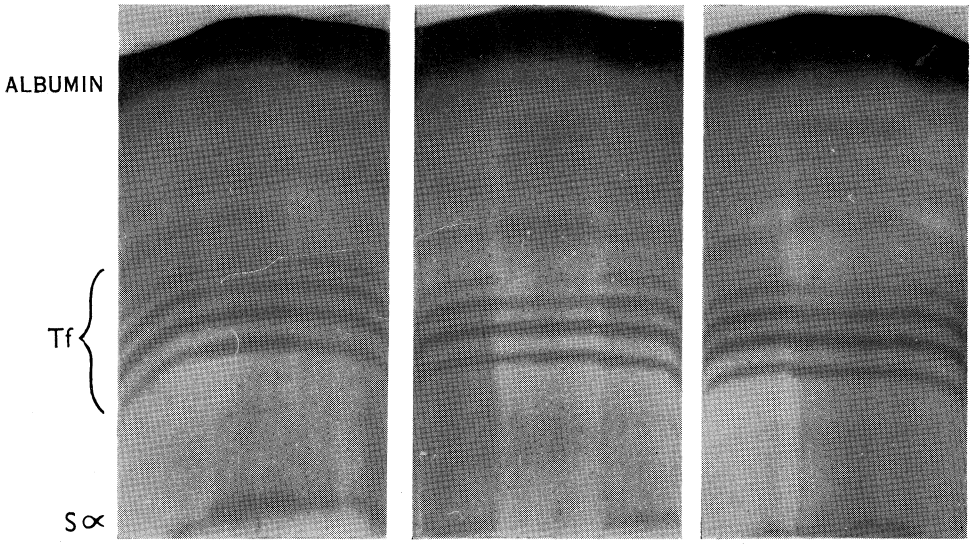
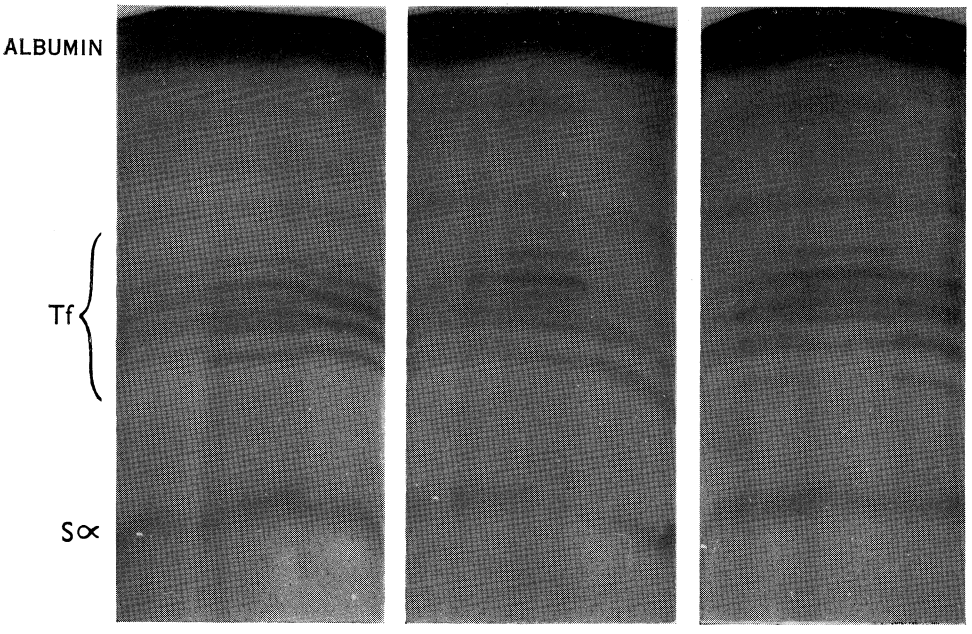
CATTLE *D* ALLELES



Portion of gels between the Sα (slow α-globulin) zone and the albumin zone showing the five transferrin phenotypes AD^1, AD^2, D^1D^1, D^1D^2, and D^2D^2.



Portion of gels between the Sα (slow α-globulin) zone and the albumin zone showing pairs of *D* alleles against an *AF* reference serum.