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An Alternative Method in Distinguishing Cattle Transferrin Phenotypes

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A modification of the method described by Kristjansson (1963) allows easier distinction of the components and position of every major cattle transferrin phenotype. The modification is based on increasing the percentage of starch (15%) and reducing the pH *of the gel buffer to 6.8. In all the experiments, when a voltage of 350 was applied, a tray of ice was placed over the starch gel for the remainder of the electrophoresis. Different cattle transferrin phenotypes from our modified electrophoresis method are composed as follows: Type A, 4 bands; D_1, 4 bands; D_1D_2, 4 bands; D_2, 4 bands; E, 4 bands; AD_1, 6 bands; AD_2, 6 bands; AE, 8 bands and sometimes 9 bands; D_1E, 6 bands and sometimes 7 bands; and D_2E, 6 bands. The position of the fourth D band is distinctly different in D_1 vs. D_2 types.*

INTRODUCTION

Smithies (1955, 1959) and Smithies and Walker (1955) demonstrated how starch gel electrophoresis could show phenotypes of serum proteins that were genetically controlled. The technique periodically is changed or modified (e.g., Poulik, 1957). Changing the technique may change the phenotype observed (Stormont, 1964). Since we detect the phenotype by useful (and often popular) but nevertheless, artificial techniques, this could change our notion of the mode of inheritance or gene action.

This report describes an improved method for the distinction of certain inherited types of transferrins, and a phenotypic change resulting from a change in technique.

Serum protein polymorphism within several species has been reviewed by Ogden

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(1961); related species have been contrasted (e.g., Braend and Stormont, 1963); and genetic analysis between species has even been made by Quinteros *et al.* (1964) and Miller (1967). The electrophoretic polymorphism of bovine transferrin (β-globulin) was first described by Ashton (1957) and Smithies and Hickman (1958).

Nine or ten codominant alleles are known, all evidently at a single locus (Ashton *et al.*, 1967). Some of the alleles are characteristic of particular breeds; for example, Tf^B and Tf^F in Zebu but not in Jersey, Hereford, or Shorthorn cattle; but other alleles, Tf^A, Tf^{D_1}, Tf^{D_2}, and Tf^E, are widespread. The major different transferrin phenotypes and their combinations are: A, AD_1, AD_2, AE, D_1, D_1D_2, D_2, D_1E, D_2E, and E. Each single (homozygous) type of transferrin migrates in starch gel electrophoresis as four discrete proteins (Ashton, 1959), heterozygotes exhibiting combinations of the four band types with different positions in the gel. But types D_1 and D_2 are often difficult to distinguish.

MATERIAL AND METHODS

One hundred fifty cattle blood samples, serum or plasma, were used. These were obtained from the "obstetrics herd," Iowa State University Veterinary Clinic (under the direction of Professor W. M. Wass), from the Woodward State Hospital herd (Mr. Harold Roberts, herdsman), and from (73) longhorns at the Wichita Mountains Wildlife Refuge, Cache, Oklahoma (Mr. Julian A. Howard, refuge manager).

The transferrin phenotypes of the samples were determined by horizontal starch gel electrophoresis with some modifications, as reported by Quinteros *et al.* (1964) and Quinteros and Muller (1967), of the method described by Kristjansson (1963) in a study of prealbumin phenotypes in pigs. In the studies by Quinteros *et al.*, the starch gels were at room temperature for $1\frac{1}{2}$ hr, followed by 30 min in the refrigerator. Briefly, that method included a starch concentration of 15% and a gel buffer of *p*H 6.8. The filter paper strips used for loading plasma were inserted 4 cm from the cathode end of the gels and at least 2 mm apart.

The paper used was 200 Streifen Filtrier papier No. 2043 or Beckman, slightly thicker than the Streifen. The paper size was 0.8×0.6 cm for each sample. After 15 min of electrophoretic run at 165 v as measured by the voltmeter of a Heath-kit regulated power supply, the paper inserts were removed and the voltage was continued 15 min more. After that, a voltage of 350 was applied for the remainder of the electrophoresis. A tray of ice was placed over the starch gel when the voltage was increased to 350. The runs were stopped and the gels were stained when the borate boundary had migrated 12 cm beyond the point of insertion.

Each of the major phenotypes was present among our samples. With this procedure, it was possible to observe bands additional to those previously distinguished by Kristjansson's method (1963), and a clearer separation of D_1 and D_2 than pictured by Kristjansson and Hickman (1965).

RESULTS AND DISCUSSION

All commonly known transferrin phenotypes are represented in Fig. 1. The distinction between D_1 and D_2 is also pictured in Fig. 2.

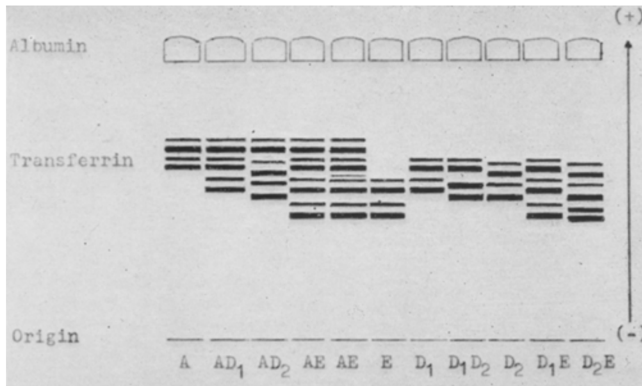

Fig. 1. Diagram of major phenotypes of transferrin in cattle.

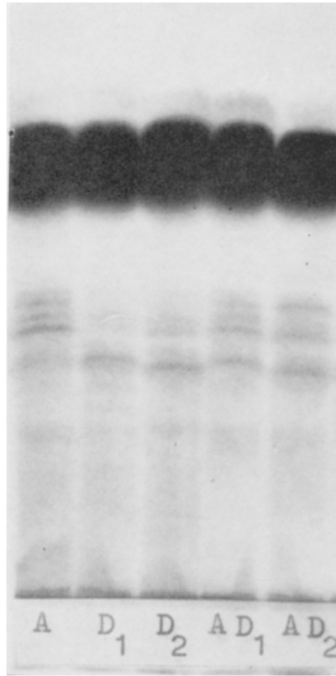

Fig. 2. The distinction between D_1 and D_2.

Table I. Different Transferrin Phenotypes

Transferrin type	Phenotype	Further description
Tf A	4 bands	The second and fourth bands are wider than the first and third ones.
Tf D_1	4 bands	The fourth or slowest band is wider than the others.
Tf D_1D_2	4 bands	The slowest band is wider than the others. The D_2 band position is clearly visualized but its exact demarcation is somewhat diffuse.
Tf D_2	4 bands	The positions are very clear. The slowest band is wider than the others and slightly slower than the slowest fourth band of type D_1.
Tf E	4 bands	Generally, the slowest band and the second one appears wider than the others.
Tf AD_1	6 bands	The second, fourth, and the slowest bands are wider than the others.
Tf AD_2	6 bands	The same as AD_1, but the D_2 band position (sixth) is slower than D_1.
Tf AE	8 bands	Generally, the second, fourth, sixth, and slowest bands are wider than the others (Fig. 1). Sometimes, a very narrow additional band appears in the D position (number 5).
Tf D_1E	6 bands	Sometimes an additional narrow band appears under the slowest D_1 band position (Fig. 1).
Tf D_2E	6 bands	In comparison with each other, the differentiation between D_1E and D_2E is easy because of the position of the slowest (fourth) D_1 or D_2 bands, D_1 being faster.

The different transferrin phenotypes are composed as shown in Table I.

The method and results we have described allow clear distinction of the transferrin types and offer a related alternative to the method of Kristjansson (1963). No change in genetic control is postulated. Evidently some transferrin phenotypes may exhibit components additional to those previously distinguished as noted by Stormont (1964) in bison. We found a 9-band AE type and a 7-band D_1E type in Holstein-Fresians. However, these extra bands have not been shown to be transferrins per se. Also, the post-albumin types are not distinguishable.

REFERENCES

Ashton, G. C. (1957). Serum protein differences in cattle by starch gel electrophoresis. *Nature* **180:** 197.

Ashton, G. C. (1959). Beta-globulin alleles in Zebu cattle. *Nature* **184:** 1135.

Ashton, G. C., Gilmour, D. G., Kiddy, C. A., and Kristjansson, F. K. (1967). Proposals on nomenclature of protein polymorphisms in farm livestock. *Genetics* **56:** 353.

Braend, M., and Stormont, C. (1963). Hemoglobin and transferrin types in the American buffalo. *Nature* **197:** 910.

Kristjansson, F. K. (1963). Genetic control of two pre-albumins in pigs. *Genetics* **48:** 1059.

Kristjansson, F. K., and Hickman, C. G. (1965). Subdivision of the allele Tf^D for transferrins in Holstein and Ayrshire cattle. *Genetics* **52:** 627.

Miller, W. J. (1967). Segregation of plasma-albumin types from a species cross. *Proc. Soc. Exptl. Biol. Med.* **124:** 1224.

Ogden, A. L. (1961). Biochemical polymorphism in farm animals. *Animal Breed. Abstr.* **29:** 127.

Poulik, M. D. (1957). Starch gel electrophoresis in a discontinuous system of buffers. *Nature* **180:** 1477.

Quinteros, I. R., Stevens, R. W., Stormont, C., and Asmundson, V. S. (1964). Albumin phenotypes in turkeys. *Genetics* **50:** 579.

Quinteros, I. R., and Muller, A. O. (1967). Grupos sanguineos y fenotipos de albumina, transferrina y hemoglobina en Equinos, P.S.C. del Haras "La Tropilla." (in press). Revista de la Facultad de Ciencias Veterinarias de la Universidad Nacional de La Plata, La Plata, Republica Argentina.

Smithies, O. (1955). Zone-electrophoresis in starch-gels. *Biochem. J.* **61**: 629.
Smithies, O. (1959). An improved procedure for starch-gel electrophoresis: further variations in the serum proteins of normal individuals. *Biochem. J.* **71**: 585.
Smithies, O., and Hickman, C. G. (1958). Inherited variations in the serum proteins of cattle. *Genetics* **43**: 374.
Smithies, O., and Walker, N. F. (1955). Genetic control of some serum proteins in normal humans. *Nature* **176**: 1265.
Stormont, C. (1964). A further comparison of bison and cattle transferrins. *Fed. Proc.* **23**: 557.