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GENETIC POLYMORPHISMS IN DAIRY CATTLE

Edward M. Wilson

GENETIC POLYMORPHISMS IN BLOOD SERUM TRANSFERRIN AND CERTAIN MILK PROTEINS OF THE COW

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GENERAL INTRODUCTION

Reports on inherited differences in certain proteins between individuals within species have appeared in the literature with increasing frequency during the past few years. These inherited differences, generally referred to as genetic polymorphisms, were discovered when techniques became available which could detect hitherto unknown small chemical differences in proteins. These genetic polymorphisms are usually caused by the action of allelic genes, and several substances with genetically controlled alternative forms have been found in numerous species.

All of the polymorphisms found in cattle have been discovered by the use of electrophoresis except for blood groups which are determined serologically. The use of electrophoresis, with a variety of supporting media, has demonstrated many previously unknown variations in proteins. Paper was used as the supporting medium up to 1955 when Smithies introduced the use of starch gel, which proved to have better powers of resolution than paper. More recently, polyacrylamide gel was introduced, which gave even better separation of some proteins than did starch gel.

To date, genetic polymorphisms have been found in cattle in such blood proteins as: the transferrins, erythrocyte antigens and hemoglobin. Evidence of genetically controlled variations in other serum proteins has been reported. The proteins are: the slow

α_2-globulin, post-albumin, haptoglobin, thread protein, acid phosphatase and alkaline phosphatase. Observations on the latter proteins are preliminary and further work is needed to confirm the hypothesis of genetic control.

Genetic polymorphisms have also been found in the following milk proteins of cattle: β-lactoglobulin, α_s- and β-casein. There is evidence to indicate that genetically controlled variation exists in γ-casein and in the whey protein α-lactalbumin; however, additional data are needed to confirm the preliminary findings which suggest genetic influence.

The ability to identify the various polymorphisms which are segregating in a population may be of importance to the geneticist and breeder in several ways. For example, (1) it is possible that the transferrin genotypes may differ in adaptive values which may be reflected in some economic characteristics of the animals. (2) The identifiable genes can be used as much needed markers in genetic studies. (3) Since several alleles are generally present in a population, they can provide a valuable check on the validity of pedigrees, and similarly can be used in the determination of twin zygosity. (4) Knowledge of the alleles present in a population and extensive study of the end products of the alleles make possible the study of gene segregation and gene action.

The present thesis is concerned with: (a) the population structure of (i) the transferrin, (ii) α_s-casein, (iii) β-casein and (iv) β-lactoglobulin loci in the Canadian herd at Station Recherches Agricoles Deschambault, Ministère de L'Agriculture, Province de Quebec,

and the Holstein and Ayrshire herds at Macdonald College. (i) The relative amounts of the electrophoretically separated transferrin bands in any one type; (ii) the constancy of the relative amounts of the transferrin bands between different animals with the same transferrin type; and (iii) the relative proportion of bands A and B in β-lactoglobulin type AB of animals in the Ayrshire and Holstein herds.

(b) To investigate the effect of transferrin types on the following production traits: milk, butterfat, protein and solids-not-fat.

(c) To investigate possible linkage between the transferrins, β-lactoglobulin, α_s- and β-casein loci.

1. HISTORICAL INTRODUCTION

1.1. Genetic Polymorphisms of Serum Transferrins or β-Globulins

The serum β-globulin which is capable of combining with iron has been termed transferrin and siderophilin because the main physiological function of this metal binding protein is to transport iron (Giblett et al., 1959; Laurell, 1961). There is a striking increase in the plasma concentration of transferrin in iron deficiency and a reduction in concentration in some disease conditions such as chronic infections and certain types of anaemia.

Smithies (1955a, 1955b) observed permanent differences in the serum proteins of normal humans and later it was demonstrated (Smithies and Walker, 1955, 1956) that these differences were simply inherited. These findings led to an investigation of the serum proteins of cattle, and the heterogeneity of serum β-globulin in this species was first reported by Hickman and Smithies (1957) and by Ashton (1957). This was followed by a full report (Smithies and Hickman, 1958) on the inherited variations in the serum proteins of cattle. The observations and propositions discussed in this report may be summarized as follows:

(1) Serum samples from more than 140 dairy cattle of the Ayrshire and Holstein breeds were examined by electrophoresis in starch gels (Smithies, 1955b) and five distinct types of serum β-globulin patterns were observed.

(2) The five β-globulin patterns contained three to five protein zones, which were designated A, B, C, D and E in decreasing order of electrophoretic mobility. These zones were observed to vary in occurrence and amount between different types.

(3) The serum type of a given animal was observed to be a fixed character of the animal during the 18 months of the investigations.

(4) A pair of monozygotic twins had the same β-globulin type.

(5) The distribution of the serum β-globulin types observed by them is shown as follows:

The distribution of serum types in the two herds*

Herd	Serum Types					Totals
	I	II	III	IV	V	
Ayrshire	10	11	10	8	3	42
Holstein	3	10	16	41	32	102

$\chi^2 = 32.0$ $P < 0.0001$

*Smithies and Hickman (1958).

The distribution of types in the two breeds differed significantly, and therefore gene frequency calculations involving two breeds would have to be made separately. However, a significant difference was not observed when a comparison was made between the distribution of serum types of the parents and those of the offspring within either breed. These comparisons suggested that the observed β-globulin differences were under genetic control.

(6) They proposed (Hickman and Smithies, 1957) the existence of a single locus at which any one of three alleles may be present, to explain the genetic control of the β-globulins. They assigned the symbol β to the locus and designated the three genes β^A, β^E and β^O. The assumption was made that the action of these genes was as follows:

The gene β^A led to the presence of protein A, B and C. When β^A was absent, protein A was absent, and the proteins B and C occurred in different proportions.

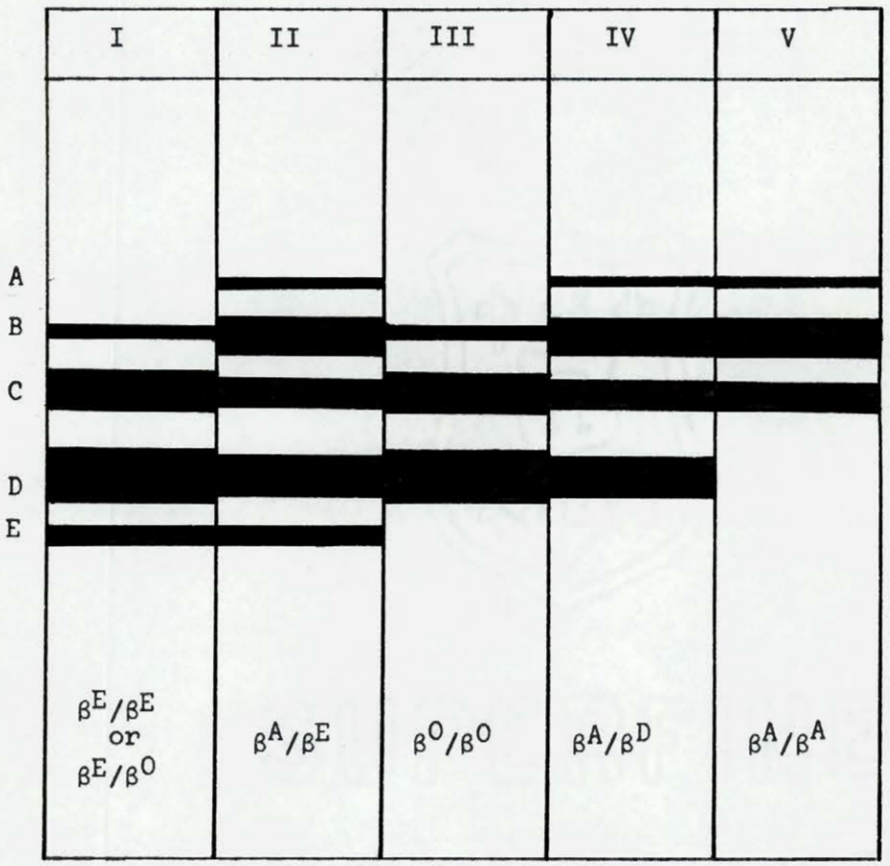
When gene β^E was present, protein E was present. If gene β^E was absent, protein E was absent.

The gene β^O was considered neutral in its effect as far as proteins A, B, C and E were concerned, in that its presence or absence did not affect the occurrence or relative proportions of these proteins.

Either of the genes β^E and β^O led to the presence of protein D in the serum, but protein D was absent in the homozygote β^A/β^A. Thus they assumed that the gene β^A was unable to cause the occurrence of protein D in the serum, although either of the genes β^E or β^O could do so.

(7) The β-globulin genotype therefore would be as follows:

SERUM TYPES



A schematic representation of the variations in β-globulins for the five serum types. The genotypes postulated are shown under the corresponding serum types. (Smithies and Hickman, 1958)

(8) Their hypothesis suggested:

(a) That the genotypes of animals with sera of types II to V are fixed. A type I animal may be homozygous β^E/β^E or heterozygous β^E/β^O.

(b) The presence of the gene β^A (which presumably cannot bring about the presence of protein D in the serum) is reflected in the

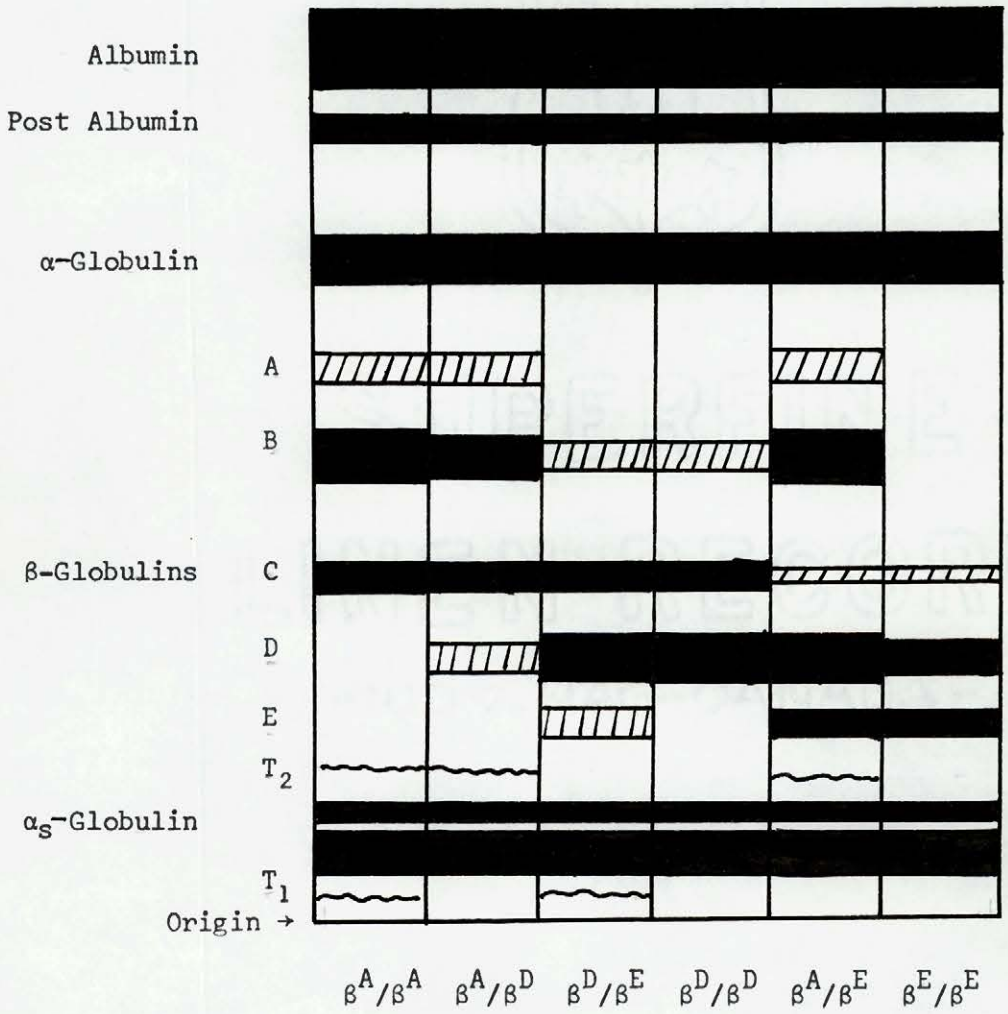
amount of protein D in sera of the types II and IV. Sera of these two types show lesser amounts of protein D than do sera of types I and III. This suggests that a "double dose" of the genes able to cause the presence of protein D (i.e., of the genes β^E and/or β^O) leads to the presence of more of this protein than does either gene singly (i.e., when accompanied by the gene β^A). Alternatively, they suggested that these observations may be equally well explained if β^A is regarded as a recessive inhibitor gene insofar as its relationship to protein D synthesis is concerned. They considered the above two interpretations for the action of gene β^A to be equivalent.

Ashton (1957) investigated by starch gel electrophoresis the serum protein differences in 12 breeds of cattle. His observations corroborated the findings of Smithies and Hickman (1958) in that five distinct types of β-globulin patterns were observed. The β-globulin phenotype of an individual animal appeared to remain constant, in that no change was observed in individual cattle on resampling over a period of twelve months. Furthermore, he noted that prolonged storage of serum at -15°C had no observable effect on the β-globulin patterns. Examination of the sera of 42 pairs of monozygous twins showed that in each case both members of the pair gave the same β-globulin pattern, again suggesting genetic control.

Ashton (1957) postulated that the β-globulin system was controlled by five pairs of linked genes, so that each of the individual β-globulins was observed in the presence of a single or "double dose" of one allele of the appropriate pair, but was absent in the presence of a "double dose" of the other allele.

Subsequently, Ashton (1958) found a sixth serum β-globulin phenotype in cattle, and proposed a genetic mechanism for the system. The six phenotypes are shown diagrammatically together with the postulated genotypes as follows:

Diagrammatic representation of cattle serum protein patterns[a]



[a]Ashton (1958).

The mating data and symmetrical appearance of the phenotypes suggested that a three allele system (genes β^A, β^D, and β^E) with no dominance was operative. The homozygotes β^A/β^A, β^D/β^D and β^E/β^E are individually recognizable. The heterozygotes are also easily recognizable, type β^A/β^D is clearly a composite of types β^A/β^A and β^D/β^D, type β^D/β^E is a composite of types β^D/β^D and β^E/β^E and type β^A/β^E a composite of β^A/β^A and β^E/β^E. Equal mixtures of the appropriate homozygous β-globulin sera are indistinguishable qualitatively from genuine samples of heterozygous serum when run electrophoretically side by side. Each of the phenotypes is thus a single genotype.

Smithies and Hickman (1958) had also postulated a three-gene theory for cattle β-globulin, although their interpretation of the expression of each gene has proved to be incorrect.

Ashton (1958) established gene frequencies for several breeds of British cattle. He pointed out that it was necessary to examine a representative cross-section of each breed in order to establish reliable gene frequency data. It is difficult to get a truly random sample by examining cattle indiscriminately, due to the preponderance of herds using one or two sires only. However, with the advent of artificial insemination, representative groups of pedigree bulls drawn from many sources have been established, and these bulls each serve many hundreds of cows every year.

The six possible β-globulin phenotypes under the proposed genetic hypothesis have been found in European breeds of cattle. In addition, Ashton (1959a) has discovered two more alleles in Zebu (Bos indicus) cattle. These have been designated β^B and β^F. This brings

the total number of alleles at this locus to five and the total number of possible genotypes to 15.

The allelic system with no dominance, as proposed by Ashton (1958) to explain the genetic polymorphisms of the β-globulins, has received wide support.

Gahne et al. (1960) analyzed serum samples from 707 animals of the Swedish Red and White cattle breed belonging to four different herds. Their results indicated a close agreement between the observed distribution of serum types and that expected under genetic equilibrium.

Hojgaard et al. (1960) confirmed Ashton's hypothesis that β-globulin types in cattle are controlled by a three-allele system and that all of the six phenotypes except β^E/β^E were found in Danish cattle.

Gahne (1961) pointed out that since β-globulin was shown to be the specific iron-binding protein of serum named transferrin (Giblett et al., 1959), that the β-globulin types of cattle be referred to as transferrin types with the symbol Tf, as used by Smithies and Hiller (1959). The latter authors used this symbol to describe the locus which determined the transferrins in humans; the capital letter superscripts being used to designate the alleles. Consequently, Gahne (1961) designated the five transferrin alleles found in cattle as Tf^A, Tf^B, Tf^D, Tf^E and Tf^F.

Gahne (1961) observed the six possible transferrin phenotypes determined by the three alleles (Tf^A, Tf^D and Tf^E) in Swedish cattle. He noted that each allele gave rise to three easily detectable zones in starch gel and that the position of the three zones was a characteristic of each allele.

Gall and Berg (1964) studied the transferrin types of cattle from two populations. One population was comprised of purebred Hereford females obtained from five breeders. The second population included foundation females of Galloway, Aberdeen Angus and Charolais X Aberdeen Angus, also obtained from five breeders. The two populations were referred to as the Hereford herd and the Hybrid herd.

The results demonstrated that the progeny data were in general agreement with the three-allele theory of inheritance, although the data suggested that offspring in the Hybrid herd carrying the Tf^E allele occurred at a lower frequency than expected.

Rausch et al. (1965) analyzed the transferrin types of 520 sire-dam-progeny combinations. Their results indicated that transferrin type was an inherited characteristic, and that a multiple allelic series of three genes, without dominance, was involved.

Carr et al. (1966) examined 2,060 serum samples from indigenous cattle in Central Africa for serum transferrin type. All fifteen phenotypes, generated by the five Tf alleles described by Ashton (1959a), were detected in this survey. The Mendelian hypothesis was tested on eight herds including at least one herd of each breed. With two exceptions, good agreement with this hypothesis was obtained. The two exceptions were traced to two bulls, a Mashona (Tf^B/Tf^D) and a Tuli (Tf^D/Tf^F), both of which contributed Tf^D to their progeny at a rate in excess of Mendelian expectation.

Recently, Kristjansson and Hickman (1965) used an improved starch gel electrophoresis technique to reveal that transferrin D in Holstein and Ayrshire cattle exists in two forms, which they designated

transferrin D^1 and transferrin D^2. Segregation data were consistent with the hypothesis that the synthesis of transferrins A, D^1, D^2 and E is under control of alleles Tf^A, Tf^{D1}, Tf^{D2} and Tf^E. Frequencies for these alleles were 0.62, 0.13, 0.16 and 0.09 respectively in the Holstein herd tested, and 0.29, 0.25, 0.21 and 0.25 in the Ayrshire herd. The differentiation of the former Tf^D allele into Tf^{D1} alleles with approximately equal frequencies increases considerably the utility of the Tf locus in parentage exclusion tests.

1.2. Effects of Transferrin Type on Certain Economic Traits

1.2.1. Transferrin type and fertility

Ashton (1959b) reported a discrepancy in the expected numbers of offspring of various transferrin types from reciprocal matings involving the Tf^A and Tf^D alleles. There were significantly more calves of types identical with dams than those with types different from their dams. Curiously enough, however, an opposite effect was found when dams possessed the Tf^E allele. These findings could not be explained on an immunological basis, since it appeared that the effect occurred in heterozygous dams as well as in homozygotes. Furthermore, Danish workers (Brummerstedt-Hansen et al., 1962) had failed to show any immunological differences between the transferrin types.

In a later study of possible relationships between transferrin type and fertility in Australian cattle, Ashton (1961) failed to confirm his original findings. He found, however, that conception rates were significantly higher in matings between homozygotes than in matings

between heterozygotes. Similar results were obtained by Ogden (1961) with English cattle. However, other workers in Czechoslovakia (Matousek et al., 1962), Denmark (Brummerstedt-Hansen et al., 1962), Sweden (Gahne, 1961), and the U.S.A. (Rausch et al., 1963; Datta et al., 1965), failed to find any significant relationship between transferrin type and fertility. Hickman and Dunn (1961) studied non-return data for bulls used in artificial insemination in New York state. Their data failed to confirm Ashton's findings of highest reproductive performance in matings between homozygotes. However, their data did support the general hypothesis of a relationship between transferrin type and fertility, since some bulls with different Tf genotypes had significantly different non-return rates and there were indications that unlike mates had a reproductive advantage. Ashton and Fallon (1962) again reported that conception rates were higher among homozygotes, especially those homozygous for the same gene. But they also reported that homozygotes were at a disadvantage in utero and that fewer than expected young were born as compared to heterozygotes. (This indeed appears to be a paradoxical finding.) Obviously there is a complex relationship between transferrin types and fertility. However, from the type and amount of data presented to date, it is very difficult to speculate on the mechanisms involved. More complete data are needed to verify some of the assumptions made. Transferrin types of sires, their mates, and all offspring born, are needed in large numbers in all possible mating combinations to clarify this apparently complex relationship.

1.2.2. Transferrin type and milk yield

Ashton (1960) studied the relationships between transferrin types and milk production. The study involved 130 bulls used for artificial insemination in Great Britain, which were classified according to their transferrin genotype. Significant differences were found between groups of bulls with three transferrin types, in the average production of their daughters, as measured by the contemporary comparison method. The average contemporary comparison values were Tf^A/Tf^A bulls, + 12.2 ± 6.9 gal.; Tf^A/Tf^D bulls, + 26.8 ± 5.4 gal.; and Tf^D/Tf^D bulls, + 38.2 ± 5.5 gal. From this data, Ashton (1960) estimated that the transferrin locus accounted for 16 per cent or more of the genetic variation in milk production. However, Robertson (1961) suggested, on the basis of all available data, that this value was too high and that 4 per cent was a more realistic estimate.

Datta et al. (1965) failed to find a significant relationship between transferrin type and production in 215 Holstein-Friesian cows. Brummerstedt-Hansen et al. (1962) stated that their studies with Danish cattle also failed to indicate any relationship. Rausch et al. (1963) studied a population of 215 Holstein-Friesians and reported that cows lacking the Tf^E allele gave more milk than cows with the allele. Further work by Ashton et al. (1964) with Australian cattle confirmed his earlier report that the transferrin locus has an influence on milk yield. The proportion of the genetic variance in milk yield due to the transferrin locus was 10.4 per cent in the Jersey breed and 6.0 per cent in the Australian Illawarra Shorthorns. This implied that the manipulation of a single gene contributing 6 to 10 per cent to genetic variance

could raise milk yield by about 5 per cent in two generations in these Australian herds. It appears that the state of knowledge on the relationship between transferrin types and milk production is similar to that on the relationship between transferrin types and fertility. More complete data are required to prove or disprove some of the assumptions made.

Ashton et al. (1964) investigated the association between transferrin type and butterfat production. The data indicated a lack of consistent evidence that transferrin type affected butterfat percentage. Transferrin type Tf^D/Tf^D cows in the Jersey breed yielded 26.4 pounds more fat than type Tf^A/Tf^A cows ($P < 0.01$). There was no significant difference between the fat yields of the Tf^A/Tf^A and Tf^D/Tf^D cows of the Australian Illawarra Shorthorn cattle. Investigations by Datta et al. (1965) failed to demonstrate an association between transferrin type and per cent butterfat in 215 Holstein-Friesian cows.

1.2.3. Transferrin type and tolerance to climatic extremes

Ashton (1958) suggested that the frequency of Tf^E may be associated with tolerance to climatic extremes. In support of this postulate, Ashton (1959a) found a high frequency of the Tf^E allele in Zebu cattle, an interesting observation in view of the well known climatic and ecological tolerance of these cattle. However, this postulate was not supported by Gahne's (1961) study with Swedish cattle.

1.3. Genetic Polymorphisms in Certain Milk Proteins of the Cow

1.3.1. Casein

Casein, the major protein in milk, has long been known to consist of several components which tend to aggregate, to interact, and to form micelles. Separation of the complex mixture by electrophoresis has presented formidable difficulties, but the problem was largely solved by Wake and Baldwin (1961), who showed that the presence of urea caused disaggregation, but not denaturation, of the casein components, leading to excellent resolution of the various constituents by starch gel electrophoresis. They were able to separate the casein complex into approximately 20 components. Incorporation of urea also improved the resolution obtained by the less complicated method of paper electrophoresis, and it was with the aid of this method that the first casein polymorphism was detected.

1.3.2. β-casein variants

Aschaffenburg (1961) discovered three variants in β-casein by the use of paper electrophoresis. He designated these variants A, B and C, in order of decreasing mobility. To explain this polymorphism, Aschaffenburg suggested that the synthesis of these proteins was controlled by three allelic genes without dominance, at a locus which he designated β-Cn, and the alleles were designated $\beta\text{-Cn}^{A}$, $\beta\text{-Cn}^{B}$, and $\beta\text{-Cn}^{C}$. On the basis of this system, six genotypes were possible, namely, A/A, B/B, C/C, A/B, A/C, and B/C, and these have all been found.

Aschaffenburg (1963) reported breed differences in the occurrence of the different forms of β-casein.

Extensive studies by Thompson et al. (1964) confirmed Aschaffenburg's genetic hypothesis. These workers used starch and polyacrylamide gel electrophoresis for genetic typing. In 168 matings of the A/A X A/A β-casein type, all of the offspring were type A/A. Sire types were deduced from progeny tests. Since the B and C forms of β-casein were relatively rare, it was not possible to make segregation analysis for mating types other than A/A X A/A. However, 13 matings of miscellaneous types produced offspring with β-casein types expected on the basis of Aschaffenburg's hypothesis.

Results of β-casein typing of 1,349 individual cows of different breeds indicated that the A allele was by far the most common in American breeds. The B allele was relatively rare in Ayrshire, Holstein-Friesian and Guernseys. Aschaffenburg (1963) has also revealed a predominance of the A allele in British breeds. The C allele was found only in Brown Swiss, Jersey and Guernsey cattle.

Peterson and Kopfler (1966) have shown that β-casein A as obtained by gel electrophoresis in alkaline buffer (one zone) will be heterogeneous (three zones), if electrophoresis is carried out in formic acid-acetic acid buffer (pH 3.0). These authors suggested that the β-caseins be renamed A, B, C, D and E according to decreasing mobility in gel electrophoresis at acid pH. β-casein typed C by electrophoresis in alkaline pH would become A, and β-casein B would remain B in the proposed nomenclature. C, D and E would be those β-caseins which are indistinguishable by alkaline electrophoresis and typed as A.

Sixty-three samples have been typed for β-casein in acid buffer, and the phenotypes observed were as follows: BC, 3; BD, 3; C, 3; CD, 33; CE, 3; D, 17; and DE, 1. Resolution of the genetic variants of α_{S_1}-caseins have not been observed in the small number of samples typed at acid pH to date. Peterson and Kopfler (1966) concluded that A type β-casein as determined at alkaline pH represented a mixture of six phenotypes. A mode of inheritance for the new types of β-casein has not yet been proposed.

1.3.3. α_S-casein variants

Thompson et al. (1962) and Kiddy et al. (1963) found that there are three genetically controlled variants in α_S-casein. The variants were designated A, B and C in decreasing order of electrophoretic mobility, and were discovered by examination of caseins isolated from milk samples from individual cows. These workers suggested that the α_S-casein variants were controlled by three autosomal alleles without dominance, $\alpha_S Cn^A$, $\alpha_S Cn^B$ and $\alpha_S Cn^C$, and that six combinations of these alleles were possible. All six genotypes have been found.

Initial studies by Thompson et al. (1962) indicated that α_S-casein variants might be genetically determined, since the first six cows whose milk showed evidence of variation were all daughters of one sire.

Kiddy et al. (1964) studied α_S-casein in order to determine the possible genetic control of variation in this protein. Milk samples from 1,378 cows from various breeds were studied. The results indicated that unlike β-casein, the B allele was by far the most common in

American cattle. The C allele was relatively rare in Holstein-Friesians and Brown Swiss, and has not been found in Ayrshires.

Analysis of family data has been complicated by the fact that the genotype of a bull cannot be determined directly. It must be deduced from a study of his daughters and their dams. The family data presented by Kiddy et al. (1964) is shown as follows, and indicates that the observed variation in α_s-casein is genetically controlled.

Deduced Genotype of bull	No. of bulls	Daughters					
		A/A	A/B	A/C	B/B	B/C	C/C
A/B	4[a]	2	71	5	68	4	..
B/B	40[b]	.	4	.	323	12	..
B/C	17[c]	.	.	.	104	110	18

[a]All Holstein.

[b]5 Ayrshire, 7 Brown Swiss, 4 Guernsey, 22 Holstein and 2 Jersey.

[c]12 Guernsey, 3 Holstein and 2 Jersey.

Gordon et al. (1965) studied the amino acid composition of α_{s_1}-casein A, B and C. They found that the variants were the same in composition, each consisting of 18 amino acids. However, they differed in the amount of some of the amino acids present.

1.3.4. β-lactoglobulin variants

Aschaffenburg and Drewry (1955) examined the whey proteins of the milk of individual cows by paper electrophoresis. They found that

β-lactoglobulin occurred in the form of two variants which differed in their electrophoretic mobility and were designated β_1- and β_2-lactoglobulin. This nomenclature was changed in 1957 by the same workers to β-lactoglobulins A and B.

The preliminary work in 1955 indicated that the type of β-lactoglobulin produced is characteristic for a given cow. At this time they examined the milk of eight sets of monozygotic twin heifers. In each set the two animals produced the same type of β-lactoglobulin. This led to the belief that the synthesis of β-lactoglobulin is genetically determined.

Further studies by Aschaffenburg and Drewry (1957) showed that the fastest-moving type, β-lactoglobulin A, occurred alone or with B. The B form was also found alone in milk from some cows. They showed that a pair of autosomal alleles without dominance was responsible for the variation, and designated the locus Lg, and the genes Lg^A and Lg^B. As with other polymorphisms, a heterozygote has two kinds of β-lactoglobulin in the milk, whereas a homozygote has only one.

The genetic hypothesis was confirmed by Plowman et al. (1959), Moustgaard et al. (1960), and by Bell (1962), who discovered a third allele which produced β-lactoglobulin C in Jerseys. This allele moved even slower than Lg^B in electrophoresis.

Kiddy et al. (1965) examined the β-lactoglobulin types of 812 individual cows of various breeds by paper and polyacrylamide gel electrophoresis. The results supported the hypothesis that β-lactoglobulins A and B are controlled genetically by codominant autosomal alleles, Lg^A and Lg^B.

They investigated milk from five heterozygous cows (Lg^A/Lg^B by moving-boundary electrophoresis, and by column chromatography to estimate the relative quantities of β-A and β-B produced by these animals. The results indicated that β-A and β-B are produced in approximately equal amounts, with β-A being slightly in excess of β-B.

Lacteal secretions were obtained from two castrated males and typed for β-lactoglobulin by means of paper electrophoresis. These animals were found to produce β-lactoglobulins B and AB consistent with their expected genotypes as deduced from pedigree analysis.

The molecules of the β-lactoglobulin variants are very similar in composition, each consisting of two identical chains of 162 amino acids (Gordon et al., 1961; Kalan et al., 1964; Piez et al., 1961). The C-chain shows only one difference from the B-chain: a histidine in place of a glutamic acid or glutamine (Kalan et al., 1964), whereas each A-chain differs in two places from the B-chain: an aspartic acid replacing a glycine, and a valine replacing an alanine (Gordon et al., 1961 and Piez et al., 1961). In spite of this over-all similarity, the molecules differ markedly in their properties.

2. MATERIALS AND METHODS

2.1. Source and Preparation of Samples

Milk and blood samples were collected from 46 Holstein-Friesian and 38 Ayrshire cows of the Macdonald College herd. Samples were also obtained from 58 Canadian cows of the Station Recherches Agricoles Deschambault. This breed (originally known as the French Canadian breed) descended from the native cattle of the provinces of Normandy and Brittany, France, and were brought into Quebec, Canada, by French settlers some 300 years ago (MacEwan, J. W. G., 1941). Because of their origin, they are related to the stock which gave rise to the Channel Island breeds. They are noted for their vigor and ability to withstand severe winter climates (Eckles, G. H., 1956). The Canadian breed is limited in distribution to the Province of Quebec, although small numbers of these cattle existed in other parts of Canada and in New York State and the New England States (St. Pierre, A., 1936).

Blood samples were collected from the external jugular vein into clean, dry 1.5 x 10 mm glass tubes. A 2 in. 18 gauge bleeding needle was used. The tubes were stoppered with a cork and placed in a horizontal position so that contact occurred between the blood sample and cork. The samples which were collected in late afternoon were left at room temperature overnight to facilitate clotting. The following morning the corks were carefully removed from the tubes in order to

withdraw the clot adhered to the cork. The fluid remaining was transferred to clean tubes and centrifuged for eight minutes at 2600 R.P.M. in an international centrifuge, model UV. The serum was poured into 1.5 x 6 ml glass vials and stored at -10°C.

2.2. Analytical Methods

2.2.1. General

Horizontal electrophoresis in polyacrylamide gel (PAE) was used for the separation of both the blood serum transferrins and the milk proteins. The apparatus was constructed of Perspex according to the design described by Aschaffenburg (1964), except that the cell was equipped with a water-cooled jacket.

2.2.2. Blood serum transferrins

The stock gel-buffer solution (pH 8.3) contained 300 ml Tris (hydroxymethylaminomethane - 0.2M), 65 ml HCl (0.2N) and 1500 ml distilled water.

The buffer solution used in the electrode chambers (pH 8.6) was prepared by dissolving 74.2 g boric acid and 16 g sodium hydroxide in a final volume of 2 liters.

The stock gel solution was prepared as follows: 90 g Cyanogum 41 (Fisher Scientific Co.) and 5 ml 3-dimethylaminopropionitrile (DMAPN) were dissolved in 1800 ml of the gel buffer solution (5% gel). This stock solution was stored in the refrigerator for subsequent use.

The gel was prepared as follows: Ammonium persulfate (0.15 g) was dissolved in 2 ml of the gel buffer solution and added to 75 ml of

the stock gel solution. The mixture was then poured into the gel former and covered for 60 minutes to permit polymerization and aging.

The serum sample was applied to the gel as described by Aschaffenburg (1964). The gel was covered with Saran Wrap and current was applied as follows: 170 v (23 ma) was applied for five minutes, the current was shut off, the sample paper inserts removed, the gel re-covered with Saran Wrap and the current again applied (170 v) for the duration of the run (8 cm as determined by movement of the borate boundary--approximately two hours). The gel was stained according to the method of Aschaffenburg (1964) except that a 1% solution of Amido Black 10B was used. The gel was de-stained by slow mechanical shaking in two changes of methanol: water: acetic acid (5:5:1v/v) and finally washing with 7% acetic acid.

2.2.3. Milk proteins

Whole milk was used for typing of β-lactoglobulin, whereas whole milk diluted 1:10 with 7M urea solution was used for typing of α_s-casein and β-casein. The samples were applied to the gel as described by Aschaffenburg (1964).

The gel buffer solution (pH 9.2) used for typing of α_s-casein and β-casein contained 112 ml Tris (0.2M), 6.5 ml HCl (0.2N) and 625 ml distilled water. The buffer solution used in the electrode chambers was the same as that described for the typing of serum transferrins.

The stock gel solution was prepared as follows: 312 g urea and 67.9 g Cyanogum 41 were dissolved in approximately 744 ml of the gel buffer solution (7% Cyanogum - 7M urea). All other electrophoretic conditions were the same as described for the serum transferrins except

that a 0.1% solution of Amido Black 10B was used to stain the milk proteins.

The electrophoretic conditions used for typing of β-lactoglobulin were the same as described for the caseins except that urea was omitted from the gel solution.

In order to establish electrophoretic patterns for the milk proteins, the following known samples supplied by Dr. M. P. Thompson (Eastern Regional Research Laboratory, Philadelphia, Pennsylvania) were used: α_{s_1}-casein B, β-casein B; α_{s_1}-casein B, β-casein AC; and β-lactoglobulin C.

2.2.4. Densitometry of the stained gels

Densitometry of the stained gels was used to study: (1) the relative amounts of the transferrin bands in any one type; (2) the constancy of the relative amounts of the transferrin bands between different animals with the same transferrin type; and (3) the relative proportion of bands A and B in β-lactoglobulin type AB of animals in the Ayrshire and Holstein herds.

A Photovolt Densicord model 542 equipped with a recorder and a Photovolt integrator model 49 was used. The integral, i.e., the area under the curve, was automatically recorded with the curves, the counts furnished by the integraph represented directly the concentration of each band.

2.2.5. Production data

Data on milk yield, butterfat per cent, per cent protein and per cent solids-not-fat (SNF) were available from the Macdonald College

herd records. All cows were milked twice daily, and the length of lactation varied from 252 to 305 days. For butterfat, protein and SNF determinations, each animal was sampled approximately once per month throughout her lactation. The figure used for computation was an average of all tests recorded for each animal to date. These figures were not available for the Canadian herd; hence samples from this herd were analyzed during the period from September 1965 to May 1966.

On a test day, proportionate samples of the night and morning milk of an individual cow were obtained by means of the "milk-o-meter" sampling device. Samples taken at the night milking were refrigerated immediately. The morning sample was added to the night sample and refrigerated until delivery to the laboratory.

All milk samples were analyzed as follows:

Crude protein.--Total nitrogen was determined by the A.O.A.C. (1960) Micro-Kjeldahl method. The percentage nitrogen was converted to percentage protein by use of the factor 6.38.

Fat.--Fat content was determined by the Babcock method (A.O.A.C., 1960).

Solids-not-fat (SNF).--Solids-not-fat were determined by the Golding plastic beads hydrometer method for the determination of SNF (Udy Analyzer Company, Inc., Pullman, Washington). The SNF content was obtained by use of the following formula:

$$SNF = 9.133 - 0.279D + 0.307F$$

where D = number of beads down

F = percentage fat

SNF = percentage solids-not-fat.

Difficulty is always experienced in assessing milk yield data for evidence of genetic effects owing to the predominant effects of feeding and management, etc. (Robertson and Rendel, 1954). To minimize these effects, the "Breed Class Average" (BCA) was used instead of total milk production. The dairy breed associations classify all registered animals into age groups, and compute the average production for each group. This is regarded as a standard (100) with which all others of similar age may be compared, and given a rating which is the "Breed Class Average."

3. RESULTS AND DISCUSSION

3.1. Electrophoresis

In preliminary studies, polyacrylamide proved to be a convenient medium for the electrophoretic separation of protein variants. The gels were easier to prepare and to manipulate than were starch gels, as they could be formed without heat treatment and could be stained without slicing of the gel. Moreover, polyacrylamide gels gave better resolution and lent themselves to simplified procedures which do not require the isolation of appropriate milk protein fractions but permit phenotyping by direct electrophoresis of whole milk.

A discontinuous buffer system in horizontal polyacrylamide gel electrophoresis (PAE) was used in preference to a constant buffer system. The principal advantages of this system were found to be (1) the length of time required for the electrophoretic run (2 hours in a discontinuous system as compared to 18 hours in a constant buffer system), (2) the protein bands obtained were sharp and resolution was good.

3.2. Serum Transferrins

To determine the transferrin type of a sample, the position of the transferrin bands must be determined. There are at least two visual methods by which this may be accomplished. The first involves the

inclusion of a control (transferrin type Tf^A/Tf^E - five bands) in all runs and a visual comparison of the position of the bands of the unknown samples with those of the control which has all five bands. The second procedure is accomplished by a visual comparison of the transferrin bands of one sample with other samples on the same gel. The relative intensity of stain between individual bands was of considerable assistance. In this study both methods were used; the second method was used routinely and the first was used whenever there was any doubt as to the classification of a given sample.

Cattle transferrins migrate in polyacrylamide gel as a series of three, four or five closely-spaced protein bands whose positions are characteristic for each allele. In each homozygous type (three bands) the fastest band was faintly stained, while the other two bands were heavily stained. In heterozygotes the fastest and the slowest bands were faintly stained and the others heavily stained. These findings are analogous to those obtained for the migration of transferrins in starch gel (Datta et al., 1965).

All animals were typed three times in order to minimize misclassification, particularly since it was observed that Tf^D/Tf^E could be misrepresented as Tf^E/Tf^E.

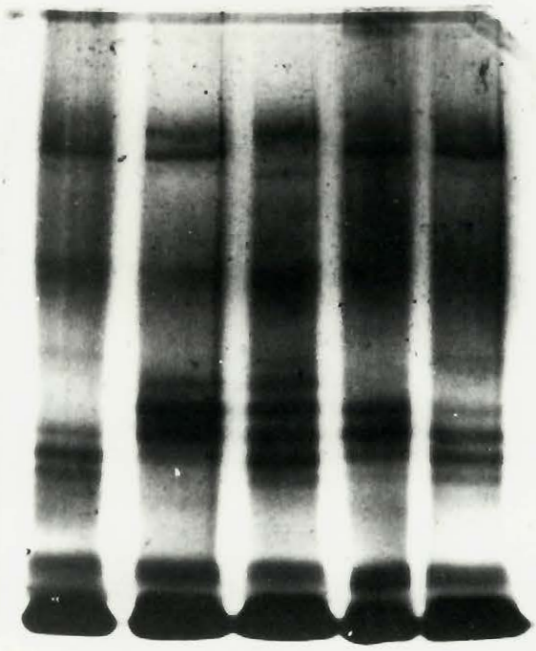
It should also be mentioned that transferrin type did not change with the age of the serum sample. The transferrin types determined on a number of serum samples which had been frozen for approximately 18 months, were identical to those determined on freshly-collected serum samples from the same animals. These observations are in agreement with those of Rausch et al. (1965).

Three transferrin alleles, Tf^A, Tf^D and Tf^E, were found in the 142 animals studied from the three breeds, namely, Holstein-Friesian, Ayrshire and Canadian. These observations are in agreement with those of Ashton (1958), who found these alleles in European breeds. Figure 1 shows a typical pattern in PAE gels of the five transferrin genotypes found in the animals studied.

Four of the six possible genotypes were present in the Holstein-Friesian cows examined, while only three types were present in Ayrshires. Tf^E/Tf^E and Tf^A/Tf^E were not observed in Holstein-Friesian and Ayrshire cattle, and in addition Tf^A/Tf^A was not present in the Ayrshire animals. A very high proportion of the animals from both breeds were typed as Tf^D/Tf^D and Tf^A/Tf^D. This agreed with the observations of Ashton (1958) and Datta et al. (1965).

Table 1 shows the distribution of transferrin types and the gene frequencies among the animals of the three herds. The gene frequencies determined by single gene counts were used to calculate the expected distribution of transferrin types, assuming the populations were in genetic equilibrium (Ashton, 1958). The distribution of genotypes in the three herds showed non-significant deviation from the expected results. This indicates that these observations are consistent with the allelic hypothesis (Smithies and Hickman, 1958; Ashton, 1958).

The frequency of the Tf^D allele in the Holstein and Ayrshire herds at Macdonald College was higher than that reported by Ashton (1958) and Smithies and Hickman (1958) for the same breeds. This could be due to the fact that 6 males sired 80 per cent of the animals studied, and by deduction from mating data, 5 of these were homozygous Tf^D and the other Tf^A/Tf^D.



A/A D/E A/E D/D A/D

FIGURE 1. —Typical polyacrylamide gel electrophoretic patterns of the five transferrin genotypes found in this study.

TABLE 1

Distribution of serum transferrin types and gene frequency in three dairy cattle breeds

Breed		Total	Distribution of Transferrin Genotypes[a]						χ^2	P(2d.f.)	Gene Frequency		
			A/A	D/D	E/E	A/D	A/E	D/E			Tf^A	Tf^D	Tf^E
Holstein-Friesian	Obs.	46	1	25	0	19	0	1	1.55	>0.30	0.23	0.76	0.01
	Exp.		2.43	26.77	0	16.08	0.21	0.70					
Ayrshire	Obs.	38	0	20	0	13	0	5	1.43	>0.30	0.17	0.76	0.07
	Exp.		1.10	21.95	0.19	9.82	0.90	4.04					
Canadian	Obs.	58	3	27	0	13	4	11	0.92	>0.50	0.20	0.66	0.14
	Exp.		2.32	25.26	1.14	15.31	3.24	10.72					

[a]For convenience the subscript Tf designated for the transferrin locus has been omitted.

Five of the six possible genotypes were present in the 58 Canadian cattle studied. The high proportion of homozygous Tf^D animals in the population studied was of interest, particularly when compared with the frequencies reported for this genotype in other dairy herds. The gene frequencies reported in Table 1 are more closely related to the frequencies reported for the Ayrshire and Guernsey breeds than to those reported for Jersey and Friesian cattle (Ashton, 1958).

3.3. Milk Proteins

Figure 2 shows a typical PAE pattern for α_{s_1}-casein (type B/B) and β-casein (types A/A and A/C), while Figure 3 shows a typical pattern for the three genotypes found in β-lactoglobulin. The distribution and gene frequencies for the milk protein genotypes are summarized in Tables 2, 3, and 4.

In the Holstein and Ayrshire cows only the B allele of α_s-casein was found; in the Canadians this was the predominant one. This observation is in accord with the reported occurrence of this allele in American breeds (Kiddy et al., 1964). The occurrence of the C allele in the Canadian breed (gene frequency, 0.09) is in closer agreement with that reported for the Jersey and Guernsey breeds than for other breeds (Kiddy et al., 1964).

It has been reported that the A allele is by far the most common one in the β-casein of American breeds, whereas the B allele is relatively rare (Kiddy et al., 1964). In this study all the animals typed from the Holstein and Ayrshire breeds were homozygous A. In the Canadian breed, the A allele was likewise the predominant one; however,

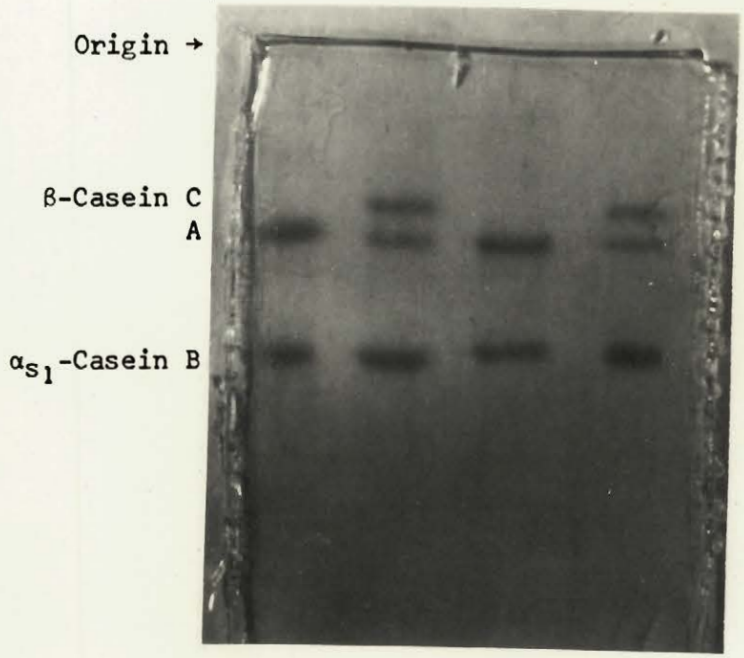


FIGURE 2.--Typical polyacrylamide gel electrophoretic patterns α_s-casein and ß-casein

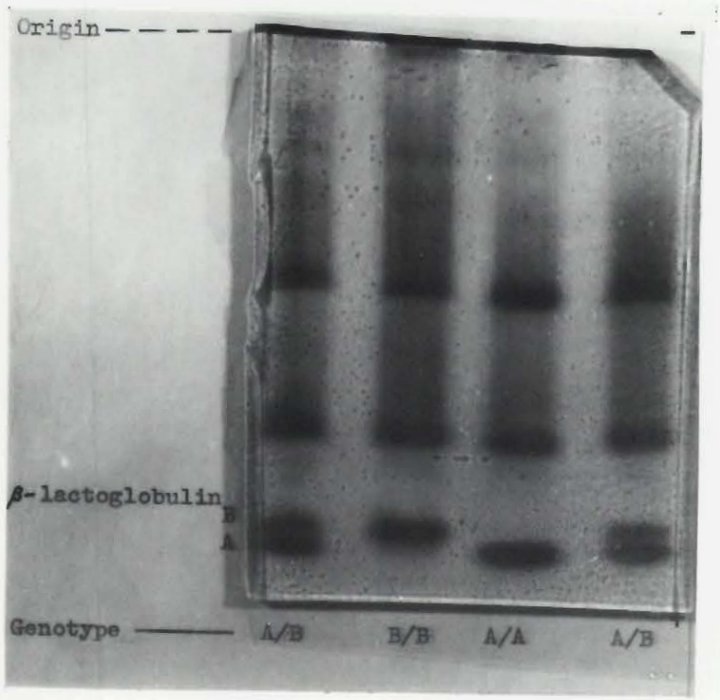


FIGURE 3.--Polyacrylamide gel electrophoretic patterns of β-lactoglobulin

TABLE 2

Distribution of β-casein types in three dairy cattle breeds

Breed		Total	β-Casein Genotypes[a]						χ^2	P(1d.f.)	Gene Frequency		
			A/A	B/B	C/C	A/B	A/C	B/C			$\beta\text{-Cn}^A$	$\beta\text{-Cn}^B$	$\beta\text{-Cn}^C$
Holstein-Friesian	Obs.	46	46	0	0	0	0	0	-	-	1.0	0	0
	Exp.		46	0	0	0	0	0					
Ayrshire	Obs.	38	38	0	0	0	0	0	-	-	1.0	0	0
	Exp.		38	0	0	0	0	0					
Canadian	Obs.	58	26	6	0	26	0	0	0.021	>0.80	0.67	0.33	0
	Exp.		26.04	6.32	0	25.64	0	0					

[a]For convenience the subscript β-Cn designated for the β-Casein locus has been omitted.

TABLE 3

Distribution of α_{s_1}-casein types in three dairy cattle breeds

Breed		Total	α_{s_1}-Casein Genotypes[a]						χ^2	P(1d.f.)	Gene Frequency		
			A/A	B/B	C/C	A/B	A/C	B/C			α_{s_1}-Cn^A	α_{s_1}-Cn^B	α_{s_1}-Cn^C
Holstein-Friesian	Obs.	46	0	46	0	0	0	0	-	-	0	1.0	0
	Exp.												
Ayrshire	Obs.	38	0	38	0	0	0	0	-	-	0	1.0	0
	Exp.												
Canadian	Obs.	58	0	50	2	0	0	6	3.25	>0.05	0	0.91	0.09
	Exp.		0	48.03	0.47	0	0	9.5					

[a]For convenience the subscript α_{s_1}-Cn designated for the α_{s_1}-Casein locus has been omitted.

TABLE 4

Distribution of β-lactoglobulin types in three dairy cattle breeds

Breed		Total	β-Lactoglobulin Genotypes[a]			χ^2 P(1d.f.)	Gene Frequency	
			A/A	A/B	B/B		Lg^A	Lg^B
Holstein-Friesian	Obs.	46	0	22	24	0.44 >0.50	0.24	0.76
	Exp.		2.65	16.78	26.57			
Ayrshire	Obs.	38	3	8	27	2.01 >0.10	0.18	0.82
	Exp.		1.23	11.22	25.55			
Canadian	Obs.	58	15	29	14	0.008 >0.90	0.51	0.49
	Exp.		15.08	28.99	13.93			

[a]For convenience the subscript β-Lg designated for the β-lactoglobulin locus has been omitted.

a high frequency (0.33) was observed for the B allele, which was in close agreement with the reported occurrence of this allele in the Jersey breed (Thompson et al., 1964). Although the C allele has been observed with low frequency in the Guernsey, Brown Swiss and Jersey breeds, it was not observed in the present study. However, this allele may yet be found in a larger population of Canadian cattle.

Both the A and the B alleles of β-lactoglobulin were observed in all three breeds examined. The three possible genotypes, $Lg^{A/A}$, $Lg^{A/B}$ and $Lg^{B/B}$ were found in the Ayrshire and Canadian breeds, but $Lg^{A/A}$ was not found in the Holstein breed. For each breed studied, the observed genotype was in close agreement with the expected genotype.

The gene frequencies reported in this study for the Ayrshire breed were in accord with those reported by Kiddy et al. (1965). However, there was some divergence in the case of the Holstein breed. In the present study a higher frequency of Lg^{B} was observed in the milk of Holsteins than the reported frequency of this allele in other studies. This could be due to the relatively few sires used in the Macdonald College herd.

In the Canadian breed, all three β-lactoglobulin genotypes were observed. The alleles Lg^{A} and Lg^{B} occurred with approximately equal frequency. This was in close agreement with the frequencies reported for β-lactoglobulin in the Jersey breed (Kiddy et al., 1965).

The data presented do not necessarily reflect the frequencies that would be found in a larger sample of the Canadian breed. However, the distribution of types even in a limited population of this breed indicated a closer relationship to the types reported for the Channel

Island breeds (Guernsey and Jersey) than to those reported for other dairy breeds.

3.4. Densitometric Studies

Figure 4 shows a densitometric plot of electrophoretically separated β-lactoglobulin. It was observed that the proportion of β-A in β-lactoglobulin AB was slightly higher (57.37%) than the proportion of β-B (42.63%) in the Ayrshire animals studied. In the Holsteins the proportion of β-A was considerably higher (62.47%) than that of β-B (37.53%). The data suggested a significant (5% level) breed difference in the production of β-A in the heterozygous β-lactoglobulin AB animals.

The observed greater proportion of β-A than of β-B is in agreement with the work of Lontie et al. (1964), who studied the β-lactoglobulins by means of agar electrophoresis at pH 8.6, followed by staining and densitometry. They observed that β-A was produced in a considerably greater amount than β-B. However, these observations are in disagreement with those of Kiddy et al. (1965), who suggested that β-A and β-B are produced in almost equal amounts by heterozygous animals, as determined by DEAE column chromatography. However, their results showed that slightly more β-A (54.42%) was produced than β-B (45.58%).

The peaks obtained by densitometry of the stained bands for the transferrin genotypes were poorly resolved, in spite of the fact that the transferrin bands were clearly resolved in the gels as judged by visual appraisal. This led to inconsistent results for any one sample. Hence it was difficult to determine quantitatively with any degree of

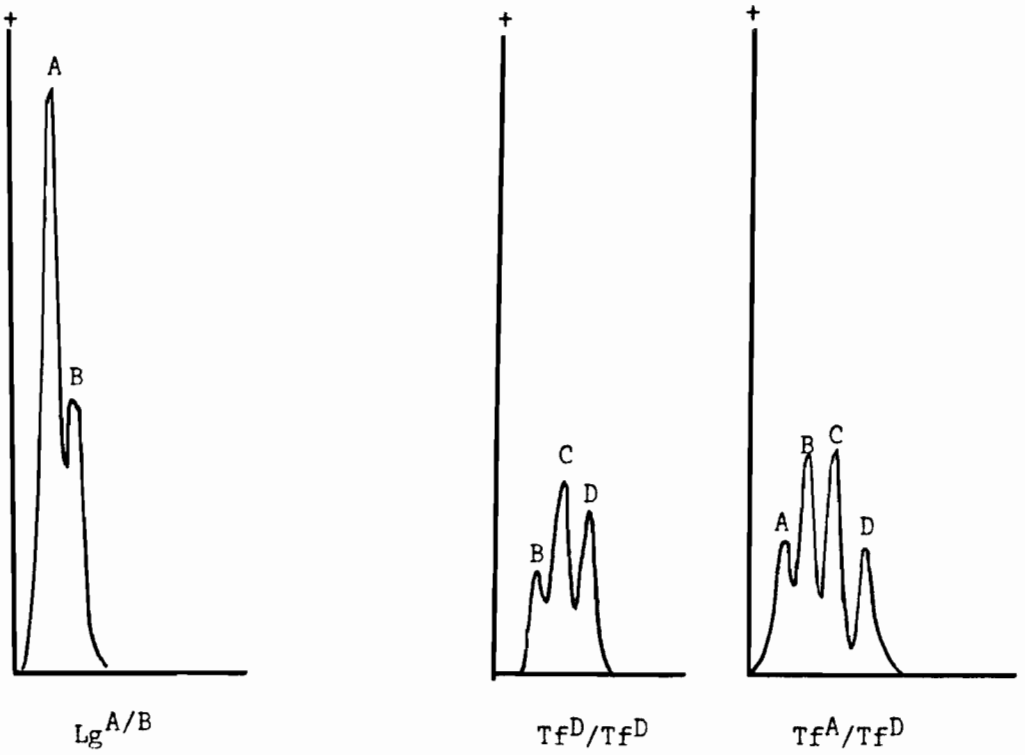


FIGURE 4.--Densitometric plots of electrophoretically separated ß-lactoglobulin and transferrins

accuracy (1) the relative amounts of the transferrin bands in any one type, and (2) the constancy of these relative amounts between animals with the same transferrin type.

The poor resolution of peaks obtained by densitometry could be due to a single factor or to a combination of factors. The transferrin bands, although sharply defined, were very close together. The refraction and/or dispersion of light in the highly transparent acrylamide gel could have been the cause of the poor resolution of peaks obtained by densitometry. Figure 4 gives an example of the best densitometric curves obtained. It was suspected that this difficulty could be overcome if the gel could be rendered opaque following removal of unbound dye. Hoogendoorn (1966) suggested that the gel could be made sufficiently opaque for densitometry by exposing it to a solution of 60% methanol for one hour.

Based on intensity of stain, the foregoing experiments indicated that in each homozygous type (three bands) the fastest-moving band was almost one-half the concentration of the other two bands. In heterozygotes (four bands) the fastest and slowest bands were present in almost the same concentration, which in turn was approximately one-half the concentration of the two inner bands. In the Tf^{A}/Tf^{E} genotype, the third band was the most concentrated, the other four bands were of approximately the same concentration, which in turn was approximately three-quarters as concentrated as the third band. The relative amount of each transferrin band appeared to be constant for all animals with the same transferrin type, judged solely by visual appraisal of the intensity of stain.

3.5. Relationships Between Transferrin Type and Milk Protein Polymorphisms

Investigations into possible linkage between the transferrins, β-lactoglobulin, α_{s_1}- and β-casein loci were carried out. It may be noted in Table 5 that insufficient variation existed in the α_{s_1}- and β-casein systems to permit linkage studies. The β-lactoglobulin loci showed some variation but the number of animals was too few to permit a linkage study. However, the data in general indicated that there might be some relationship between the Tf^D allele and the Lg^B allele.

A study involving larger numbers of animals exhibiting each of the transferrin types might possibly demonstrate a relationship between transferrin type and the milk protein variants.

Research by Thatcher and Kiddy (1965) indicated that the β-lactoglobulin and blood group J loci were linked with 20% or more recombination.

3.6. Transferrin Type and Production Data

Table 6 gives the transferrin genotype and the means within each breed for milk yield (presented as Breed Class Average - BCA), butterfat percentage, protein percentage, and solids-not-fat (SNF) percentage. The statistical analysis of the data involved the individual values for each cow.

TABLE 5

Transferrin types and milk protein polymorphisms

Breed	No. of Cows	Transferrin Genotype	α_s-Casein Genotypes						β-Casein Genotypes						β-Lactoglobulin Genotypes		
			A/A	A/B	A/C	B/B	B/C	C/C	A/A	A/B	A/C	B/B	B/C	C/C	A/A	A/B	B/B
Holstein-Friesian	1	A/A	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0
	25	D/D	0	0	0	25	0	0	25	0	0	0	0	0	0	7	18
	19	A/D	0	0	0	19	0	0	19	0	0	0	0	0	0	14	5
	1	D/E	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1
Ayrshire	20	D/D	0	0	0	20	0	0	20	0	0	0	0	0	2	4	14
	13	A/D	0	0	0	13	0	0	13	0	0	0	0	0	1	2	10
	5	D/E	0	0	0	5	0	0	5	0	0	0	0	0	0	2	3
Canadian	3	A/A	0	0	0	3	0	0	1	2	0	0	0	0	0	1	2
	27	D/D	0	0	0	24	3	0	13	12	0	2	0	0	5	15	7
	13	A/D	0	0	0	13	0	0	5	6	0	2	0	0	6	5	2
	4	A/E	0	0	0	3	0	1	0	3	0	1	0	0	2	2	0
	11	D/E	0	0	0	7	3	1	8	2	0	1	0	0	2	2	0

TABLE 6

The transferrin locus and production traits

Breed	Genotype	No. of Cows	B.C.A.[a]	Mean % Butterfat	Mean % Protein	Mean % S.N.F.[b]
Holstein-Friesian	D/D	25	150.38	3.71	3.08	8.29
	A/D	12	147.83	3.57	3.12	8.49
Ayrshire	D/D	19	153.05	3.87	3.23	8.50
	A/D	9	142.11	4.06	3.30	8.56
Canadian	D/D	21	146	4.66	3.75	8.50
	A/D	9	142	4.35	3.70	8.63
	D/E	9	139.88	4.55	3.66	8.60

[a]Breed Class Average.

[b]Solids-not-fat.

3.7. Transferrin Type and Butterfat Production

The differences between breeds for butterfat per cent were non-significant. This finding is in agreement with the observations of Ashton (1960) and Ashton et al. (1964) on the relationship between transferrin genotype and butterfat production.

3.8. Transferrin Type and Milk Production

Ashton (1960) found a significant association between milk yield and transferrin genotypes. He stated that about 17% of the total genetic variation in milk yield was due to the transferrin locus.

In a later study Ashton et al. (1964) concluded that the proportion of the genetic variance in milk yield due to the transferrin locus was 10.4% in the Jersey breed and 6.0% in the Shorthorn breed.

Ogden (1961) cited an unpublished study that confirmed the association between transferrin type and milk yield in the Ayrshire breed, but not for the other breeds, and estimated that only about 4% of the total genetic variation in milk yield was influenced by the transferrin locus.

Brummerstedt-Hansen et al. (1962) in their study with Danish cattle, could not demonstrate a relationship between transferrin type and milk yield.

Datta et al. (1965) found a non-significant relationship between transferrin type and milk production in Holsteins. However, they observed that type Tf^D/Tf^D cows showed slightly higher average milk yields than types Tf^A/Tf^A and Tf^A/Tf^D cows.

In the present study a significant relationship was not found between transferrin type and milk production (BCA); in both the Ayrshire and Canadian animals studied, however, type Tf^D/Tf^D cows in both the Ayrshire and Canadian herds showed slightly (not significant) higher average milk yields than type Tf^A/Tf^D cows (Table 6).

This study indicated that there may, in fact, be a relationship between transferrin type and milk production, but more complete data will be required for confirmation. Transferrin types of sires, their mates, and all offspring born are needed in large numbers in all possible mating combinations to establish this apparent relationship.

3.9. Transferrin Type, Protein and Solids-Not-Fat (SNF) Production

A significant difference between transferrin genotype and per cent protein was not observed in all three breeds examined, suggesting that there may be no relationship between transferrin type and protein production.

Similarly, a significant difference was not found between transferrin types and SNF production in the Ayrshire and Canadian animals studied. However, type Tf^A/Tf^D Holsteins had a significantly higher (1% level) SNF content in their milk than type Tf^D/Tf^D cows.

The above results indicate that again there may be a relationship between transferrin type and SNF production. However, the numbers involved in this study were too limited to provide conclusive results.

4. SUMMARY

The blood serum transferrins, α_s-casein, β-casein and β-lactoglobulin variants in three herds representing three dairy cattle breeds were studied by means of horizontal polyacrylamide gel electrophoresis in a discontinuous buffer system.

(1) Five transferrin phenotypes were observed in the Canadian herd, four in the Holstein-Friesian, and three in the Ayrshire herd.

(2) Gene frequencies were calculated for each herd, and were used to calculate the expected distribution of transferrin types. The distribution of genotypes in the three herds, showed non-significant deviation from the expected results. This indicated that these observations were consistent with the allelic hypothesis.

(3) In the Holstein and Ayrshire herds only the B allele of α_s-casein was found. This was the predominant allele in the α_s-casein of the Canadian herd, but the C allele was also present (gene frequency, 0.09).

(4) All the animals from the Holstein and Ayrshire breeds typed for β-casein were found to be homozygous A. In the Canadian breed, the A allele was likewise the predominant one; however, a high frequency (0.33) was observed for the B allele, which is in close agreement with the reported occurrence of this allele in the Jersey breed.

(5) Both the A and the B alleles of β-lactoglobulin were observed in all three breeds examined. The three possible genotypes,

$Lg^{A/A}$, $Lg^{A/B}$ and $Lg^{B/B}$, were found in the Ayrshire and Canadian breeds, but $Lg^{A/A}$ was not found in the Holstein breed. For each breed studied the observed genotypes were in close agreement with the expected genotype.

(6) It was observed by densitometry of the stained gels, that the proportion of β-A in β-lactoglobulin AB was slightly higher (57.37%) than the proportion of β-B (42.63%) in the Ayrshire animals studied. In the Holsteins the proportion of β-A was considerably higher (62.47%) than that of β-B (37.53%). The data suggested a significant (5% level) breed difference in the production of β-A in the heterozygous β-lactoglobulin AB animals.

(7) Due to poor resolution of the transferrin peaks by densitometry, it was difficult to determine quantitatively with any degree of accuracy the relative proportions of the transferrin bands in any given type, and the constancy of these relative proportions between animals with the same transferrin type. However, based on intensity of stain, the results indicated that in each homozygous type (three bands) the fastest-moving band was almost one-half the concentration of the other two bands. In heterozygotes (four bands) the fastest and slowest bands were present in almost the same concentration which in turn was approximately one-half of the concentration of the two inner bands. In the Tf^{A}/Tf^{E} genotype, the third band was the most concentrated; the other four bands were of approximately the same concentration, which in turn was approximately three-quarters as concentrated as the third band. The relative amount of each transferrin band appeared to be constant

for all animals with the same transferrin type, judged solely by visual appraisal of the intensity of stain.

(8) Insufficient variation existed in the α_{s_1}- and β-casein systems to permit linkage studies between the transferrins, α_{s_1}- and β-casein loci. The β-lactoglobulin locus showed some variation, but the number of animals was too limited to permit a linkage study. However, the data in general indicated that there may be some relationship between the Tf^D allele and the Lg^B allele.

(9) The possible relationship of transferrin type to milk yield, butterfat percentage, protein percentage and solids-not-fat (SNF) percentage was studied. The results were in general agreement with those of other workers, indicating a possible relationship between milk yield and transferrin type and no relationship with butterfat percentage. The present study failed to demonstrate a relationship between transferrin type and protein percentage, but a relationship between transferrin type and SNF production in the Holstein breed was indicated.

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