INHERITED BIOCHEMICAL POLYMORPHISMS AND THEIR ASSOCIATION WITH PRODUCTION IN DAIRY CATTLE

by

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BIOCHEMICAL POLYMORPHISMS AND PRODUCTION IN CATTLE

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INHERITED BIOCHEMICAL POLYMORPHISMS AND THEIR ASSOCIATION WITH PRODUCTION IN DAIRY CATTLE

SUMMARY

Two biochemical polymorphisms, one involving the serum protein transferrin and the other the J-Oc blood group system, have been studied in dairy and other cattle in South Australia. The relationship between these systems and various production characteristics has been examined in two pure bred dairy cattle populations.

The published evidence for an association between transferrin type and fitness is conflicting. Some of the anomalous data may be the result of difficulties in accurate determination of parentage. To obviate such errors, 337 dam-foetus pairs were collected from an abattoirs and their transferrin type determined. Analysis of these data has shown that transferrin type has no observable effect on fitness and no effect on maternal-foetal compatibility. Such dam-foetus pairs provide incomplete family data and these are in complete agreement with the hypothesis that the transferrin variants are controlled by a number of alleles at a single locus.

The transferrin genotype of the sires of 51 half-sib families has been inferred from their progeny and there is evidence for an appreciable misclassification of pure-bred animals in South Australian herds.

A bull at the Artificial Breeding Centre in South Australia was found to have an aberrant transferrin DE phenotype where the slowest E zone migrated slightly faster than normal. The transferrin types of 22 offspring of this bull have shown that the pattern is not inherited in the same way as the other known variants and may be controlled by another locus.

Sprague (1958a) proposed that the J-Oc phenotypes are controlled by a series of alleles at a single locus. As a result of observations on the presence of J and Oc substances in both the serum and seminal plasma of bulls and in the serum of the abattoirs population of cows, alternative models have been proposed and these are discussed in relation to Sprague's (1958a) data. It has been reported in the literature that certain electrophoretic variants of the enzyme alkaline phosphatase show an association with the serologically related blood groups, ABO, R-O-i and J-Oc of man, sheep and cattle respectively. No such association with J-Oc could be demonstrated in the abattoirs population.

Transferrin patterns of J blood group types were studied in foetuses of different ages. It was shown that a given transferrin genotype has a variable expression in the foetus and approaches the adult phenotype with increasing age.

J and Oc substances were examined in foetal and immediately post-natal sera. A significant correlation was found between the titre of the two substances in JOc foetuses and between the

titre of Oc substance and foetal length in Oc foetuses. The bearing of such observations on the proposed models for the control of production of J and Oc substances is discussed.

Transferrin, serum albumin and J-Oc in Banteng cattle (*Bibos banteng*), Asiatic buffalo (*Bubalus bubalis*) and the Eland were examined and the findings are discussed in relation to the taxonomic relationships between these species and domestic cattle.

The relationships between seven production parameters and the transferrin and J blood group types were examined in a Friesian and a Jersey cattle population. In the Friesian population, cows of transferrin type A produced milk with a significantly higher fat percentage than those of type D. There was no significant association between the remaining production parameters and the phenotypes considered. These findings are discussed in relation to other published work, to their genetic and practical significance and to the role of artificial selection in the maintenance of the transferrin polymorphism.

DECLARATION

I declare that this dissertation comprises my own work, except where specifically stated to the contrary, and that it is not substantially the same as any dissertation which has already been submitted to any other University.

L. F. Bailey

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INTRODUCTION

The principal application of genetics to animal improvement has been in the field of quantitative genetics, and over the last 30 years techniques developed for the estimation of the genetic components of the variance of quantitative production characters have been applied to a number of domestic animals. Initially, quantitative genetics had its greatest impact on the breeding of animals with short generation times and relatively large family size like poultry and swine: however, with the increasing use of artificial insemination for dairy cattle breeding, it has been possible to apply these techniques to the breeding and selection of these animals with some success (Robertson and Rendel, 1954; Ashton, 1960b). The statistical approach to animal improvement is thus well established.

In contrast with the development and application of quantitative genetics has been the concurrent description of genetic variation in the red-cell antigens and body fluid proteins of animals. Immunological and electrophoretic variants of the polysaccharides and proteins of animal tissues and fluids are generally attributable to single gene differences between animals and the description of these variants has been the subject of active interest, especially in the field of electrophoretic protein variants in the last ten years. This has been mainly due to the development and use of new techniques. Thus, whereas quantitative genetic theory has reached the stage of application to economic situations, the study of the discrete inheritance of biochemical polymorphisms is still mainly descriptive. It is a simple matter to detect and describe a biochemical polymorphism but it requires far more work to detect and elucidate the forces maintaining the polymorphism in the population. As a consequence, very few workers have attempted to take the study of biochemical polymorphisms beyond the descriptive phase.

In domestic animals there have been attempts to find associations between these simply inherited variants and quantitative production and fertility attributes with varying degrees of success but with sufficient promise, especially in poultry, to provide incentive for further studies. Certainly, with the present trend towards thinking in terms of only a few loci controlling a large proportion of the genetic variation of quantitative characters (Robertson, 1966), it seems likely that some detectable single gene differences manifested as protein polymorphisms may have a far greater effect on animal production characters than once thought possible.

Besides being potentially suitable for use in animal improvement, biochemical polymorphisms in animals are being used increasingly to study the evolutionary relationships between species and the phylogenic relationships between breeds and races of animals. Taxonomic problems which for some time were the subject of controversy, in some cases have been resolved by studying serum protein patterns and erythrocyte antigens (Stormont *et al.*, 1961). The dairy cattle industry in Australia is our largest single industry in terms of man power employed, and is based almost entirely on animals derived from British breeds but with

some history of introduction of tropical breeds. Furthermore the
industry is undergoing a phase in development in which government spon-
sored schemes are taking over part of the work of animal improvement on
a State basis, the activities of the state bodies being coordinated by
national committees. The introduction of artificial insemination
services and progeny testing schemes provide the opportunity to obtain
production data from a large number of animals and to collect more
extensively and systematically, samples of blood to determine many of
the simply inherited characters recently reported. These two factors
have facilitated a study on dairy and other cattle in South Australia.
Consideration of both quantitative performance parameters and simply
inherited blood-group differences in this study has enabled a description
1 of two cattle populations with respect to a number of biochemical poly-
morphisms and in addition to determine the relationship between the
2 polymorphisms and dairy cattle production parameters. Furthermore, it
has been possible to test the hypotheses that the polymorphisms are due
3 to the segregation of alleles at a single locus and are selectively
balanced, and to examine the degree of relationship between two cattle
4 species. In addition, the nature of the material collected facilitated
5 some observations on the prenatal development of certain blood-group
substances.

The thesis is divided into two sections. Section A is concerned with the analysis of family and population data for the transferrin and J blood group polymorphisms; with the prenatal development of blood-group substances and with the phylogenic relationships between cattle species. Section B is devoted to an investigation of the possible association of transferrin type and J blood-group type with production characters in dairy cattle.

SECTION A

FAMILY AND POPULATION DATA FOR THE TRANSFERRIN, J AND ALKALINE PHOSPHATASE POLYMORPHISMS

I. LITERATURE REVIEW

a. Transferrin

1. General

Zone electrophoresis in starch gel was first described by Smithies (1955). It allowed the resolution of plasma proteins into distinct zones or bands, giving separation not previously achieved by classical methods of electrophoresis. This enabled Smithies (1957) to detect variation in a component of the beta-globulin fraction of human plasma. It was subsequently shown that the difference between the variant type (D_1) and the normal type (C) was inherited (Horsfall and Smithies, 1958) and that the variant beta-globulin was transferrin or siderophilin, the iron-binding protein of the plasma (Smithies and Hiller, 1959; Giblett et al., 1959). Since that time at least 14 human transferrin variants have been described (Parker and Bearn, 1962).

Starch gel electrophoresis and Fe^{59} autoradiography have now been used to examine the serum of a large number of animal species and inherited transferrin variation has been found in most domestic animals as well as many non-domestic species. The results of a number of studies are summarized in Table 1. At present, the bison (Braend and

TABLE 1

A SUMMARY OF THE TRANSFERRIN VARIATION DESCRIBED IN VERTEBRATES

Species	Gene Symbols*	Multiple Zones	References
Domestic cattle (*Bos taurus* and *Bos indicus*)	A, B, D_1, D_2, E, F, H, G_{Kenya}, $G_{South\ Africa}$	6	Ashton (1957), Hickman and Smithies (1957) Ashton (1959a) Ashton (1958d) Kristjansson and Hickman (1965) Ashton (1965a), Osterhoff and Van Heerden (1964a), Ashton and Lampkin (1965), Sartore and Bernoco (1966), Makarechian and Howell (1967)
Domestic sheep (*Ovis aries*)	A, B, C, D, E (F, A, H, J, K) G, N, L, P, F	2	Ashton (1958a), Ashton and Ferguson (1963), Khattab, Watson and Axford (1963), Nasrat and Osterlee (1965)
Domestic goat (*Capra hircus*)	A, B	2	Ashton and McDougall (1958) Millson and Pattison (1961)
Horse (*Equus caballus*)	D, H, M, O, R, F	2	Ashton (1958b), Schmid (1962), Braend and Stormont (1964), Gahne (1966)
Donkey & Zebra (*Equus asinus* and *Equus burchelli*)	Dd, I, J, Dz	2	Kaminski (1964), Osterhoff (1966b)
Pig (*Sus scrofa*)	A, B	3	Ashton (1960a), Kristjansson (1960a,b)
Dog (*Canis familiaris*)	M, F, S	3	Braend (1966)
Cat (*Felis domesticus*)	A, B	3	Bailey (unpublished)
Monkeys, Primates, Man	Various	2	Lai and Kirk (1960), Blumberg (1960), Goodman and Poulik (1961), Beckman *et al* (1961), Boyer and Young (1960), Buettner-Janusch (1961, 1963), Lange and Schmitt (1963), Parker and Bearn (1962)

Table 1 - page 2

Species	Gene Symbols*	Multiple Zones	References
Mouse (*Mus musculus*)	A, B	3	Cohen (1960), Shreffler (1960), Cohen and Shreffler (1961), Ashton and Braden (1961)
Ground Squirrel (*Spermophilus* spp.)	Not given	Not given	Nadler and Hughes (1966)
Fox (*Vulpes vulpes*)	Not given	Not given	Kaminski and Balbierz (1964) Kaminski *et al* (1966)
Reindeer (*Rangifer tarandus tarandus*)	C, E, G, I, K, M, A, H	2	Gahne and Rendel (1961) Braend (1964)
Red deer (*Cervus elaphus*)	a, b, c, d, e	1	Lowe and McDougall (1961)
African Antelopes (*Taurotragus oryx* and *Gazella* spp.)	A, B, C, D, E, F, G, H, J	2	Ashton and Carr (1965)
Red Kangaroo (*Megaleia rufa*)	1, 2	4	Cooper and Sharman (1964), Bailey and Lemon (1966)
Grey Kangaroo (*Macropus giganteus*)	A, B	3	Kirsch, Poole and Sharman (pers. comm.), Bailey (unpublished)
Brush tailed Possum (*Trichosurus vulpecula*)	A, B, C	2	Hope (pers. comm.), Kirsch (pers. comm.)
Fat tailed marsupial mouse (*Smynthopsis crassicaudata*)	A, B	2	Hope (pers. comm.)

Table 1 - page 3

Species	Gene Symbols*	Multiple Zones	References
Weddell seal (*Leptonychotes weddelli*)	F, S	2	Shaughnessy (pers. comm.)
Fur seal (*Arctocephalus forsteri* and *A. tasmanicus*)	A, B, C	3	
Pigeons and Doves (*Columba livia* and *C. guinea*)	L_1, L_2, G_1, G_2	4	Mueller *et al* (1962)
Domestic fowl (*Gallus gallus*)	a, b	2	Ogden *et al* (1962)
Geese (*Anser* spp. *Branta* spp.)	Not given 8 patterns	2	Baker and Hanson (1966)
Ring-necked Pheasant (*Phasianus colchicus*)	S, F	2	Baker *et al* (1966)
Snakes (9 species)	Not given	1	Dessauer *et al* (1962)
Tree goanna (*Varanus varanus*)	A, B	3	Bailey (unpublished)
Toad (*Bufo terrestris*)	Not given 4 patterns	2	Dessauer *et al* (1962)

Table 1 - page 4

Species	Gene Symbols*	Multiple Zones	References
Cod (*Gadus morhua*)	E, D, C, C', B, A, A'	1	Moller and Naevdal (1966)
Coalfish (*Gadus virens*)	B, A, A', A_1, A_1'	1	
Pollack (*Gadus pollachius*)	B, A	1	
Whiting (*Gadus merlangus*)	B, A	1	
Herring (*Clupea harengus*)	B, A	1	
Sprat (*Clupea sprattus*)	B, A_1, A_2	1	
Plaice (*Pleuronectes platessa*)	F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T	1	Ligny (1966)
Carp (*Cyprinus carpio*)	A, B, C	1	Greyssel *et al* (1966)
Eel (*Anguilla anguilla*)	A, B, C	1	Fine (1966)

* All locus symbols are *Tf* with the exception of the mouse where it is *Trf*.

Stormont, 1963), the Norwegian elk (Braend, 1962), the mink (Gahne, 1964), the albino rat, the Rottnest Island wallaby (Ezekiel _et al._, 1963) and two species of toad (Fox _et al._, 1961) are the only species in which extensive studies have revealed no transferrin polymorphism.

Few of the transferrin polymorphisms in Table 1 have been investigated intensively and large family data are not generally available. However, where family data have been examined they are in agreement with the hypothesis that the differences between transferrin types are determined by a series of alleles at a single locus. Very few exceptions have been reported (Smithies and Hickman, 1958; Gahne, 1961) and all of these are attributed to errors in parentage records.

In different species the transferrin genes are responsible for the production of a set of two, three, four or five proteins electrophoretically separable in starch gel (Table 1). In most species studied each transferrin allele determines the production of two protein bands, one major and one minor (primates, sheep, goats, horses, antelopes, reindeer); however, in mice, pigs and cats the comparable number is three, in kangaroos four (Bailey and Lemon, 1966) and in cattle six (Makarechian and Howell, 1967). Thus, the number of protein bands in a set is characteristic of a species and the substitution of one allele for another causes a stepwise change in all the bands of a set. Since there is no apparent dominance, the phenotype of each homozygous parent is detectable in a heterozygote. Thus, a heterozygote has twice the number of bands as a homozygote and the intensity of these bands is about half that found in the homozygote.

Multiple protein zones determined by a single gene are common in protein polymorphisms described in the literature. Thus, cattle post-albumins and rabbit red cell esterases are inherited as a zone duo (Gahne, 1963a; Grunder et al., 1965) and horse pre-albumin and serum esterases as a zone trio (Gahne, 1965). The explanation of multiple zones varies, and in very few cases the hypotheses have been experimentally tested. There are three types of hypothesis proposed to explain multiple zones:

(i) Interaction with gel buffer, as proposed for fowl conalbumin in boric acid (Parker and Bearn, 1963; Williams, 1962) and for mouse transferrin in tris-maleate (Hope and Bailey, unpublished).

(ii) Multimer formation as with rabbit red cell esterases (Grunder et al., 1965), baboon lactate dehydrogenase (Syner and Goodman, 1966) and maize esterase (Schwartz et al., 1965).

(iii) Enzymic attachment of charged carbohydrate moieties in varying proportions as proposed for human transferrin (Parker et al., 1963; see also Jamieson, 1964).

Even though each explanation may be valid for the situation described, it is apparent that no single explanation can explain all multiple protein zones. The five multiple transferrin zones found in human foetal serum are apparently induced by a deficiency in an enzyme which usually attaches four negatively charged carbohydrate moieties to the transferrin molecule (Parker et al., 1963). This may be a general explanation for multiple transferrin zones in other species.

2. Transferrins in cattle

Transferrin polymorphism in cattle was first described by Ashton (1957) and Hickman and Smithies (1957). On the basis of 150 matings involving the five observed transferrin (then known generally as beta-globulin) phenotypes I-V corresponding to what are now A, AD, DE, D and AE, Ashton proposed that the data might be accounted for by five pairs of linked loci. This hypothesis was probably based on the observation that the maximum number of bands observed in a phenotype was five (type V). Interpretation of these early observations on cattle transferrin was made difficult by the number of multiple zones and the absence of $\underline{Tf}^E$ homozygotes from the sampled animals. Smithies and Hickman (1958) proposed a series of three alleles ($\underline{\beta}^A$, $\underline{\beta}^E$, $\underline{\beta}^O$) at the transferrin locus to explain the same five phenotypes designated V, IV, I, III and II respectively, where $\underline{\beta}^O$ was an inert allele. Thus, individuals with pattern II (AE) were $\underline{\beta}^A\underline{\beta}^E$, those with pattern III (D) $\underline{\beta}^O\underline{\beta}^O$, those with IV (AD) $\underline{\beta}^A\underline{\beta}^O$ and those with V (A) $\underline{\beta}^A\underline{\beta}^A$. Individuals with pattern I (DE) however, could be either $\underline{\beta}^E\underline{\beta}^E$ or $\underline{\beta}^E\underline{\beta}^O$. Again, the failure to recognize the zone trio as the inherited unit and the absence of transferrin type E from the sample prevented interpretation of the data in the sense that is now commonplace for protein polymorphisms. The hypothesis of Smithies and Hickman (1958) required little modification by Ashton (1959a) to obtain a working hypothesis to explain the cattle observations and it has been frequently invoked, often on very slender evidence, to account for the genetic control of all transferrin and indeed all protein polymorphisms.

In 1958(d) Ashton attributed the transferrin variation in cattle to 3 alleles $\underline{\beta}^A$, $\underline{\beta}^D$ and $\underline{\beta}^E$ at the transferrin locus and in (1959(a) he described variants B and F ($\underline{\beta}^B/\underline{\beta}^B$ and $\underline{\beta}^F/\underline{\beta}^F$) in zebu cattle. Since that time the locus symbol has been changed to <u>Tf</u> (Gahne, 1961), transferrin D has been subdivided into D_1 and D_2 (Kristjansson and Hickman, 1965; Ashton, 1965a), Gahne (1961) and Osterhoff and van Heerden (1964a) have described transferrin G ($TfG_{South\ Africa}$), Ashton and Lampkin (1965a) have found a new variant in East African zebu cattle which they also called G but which has been called A_1 (Jamieson, 1965) and G_{Kenya} (Ashton <u>et al</u>., 1966) and Sartore and Bernoco (1966) have described a variant faster than A which they called H. The relationship between A_1 (G) of Ashton and Lampkin (1965a) and this type is not known but in Figure 1 it is placed nearer the cathode. Besides these regular variants a number of workers have found transferrin patterns which do not conform to the standard phenotypic pattern (Gall and Berg, 1964; Sartore and Bernoco, 1966). Figure 1 shows the relative mobility of the known cattle variants. In this figure, transferrin homozygotes are shown with four bands after Jamieson (1965). Early workers recognized only three bands (see p.8), however with improved techniques four bands have been shown to have iron-binding ability (Neethling and Osterhoff, 1966), and recently Makarechian and Howell (1967) have claimed that there are six bands in homozygotes.

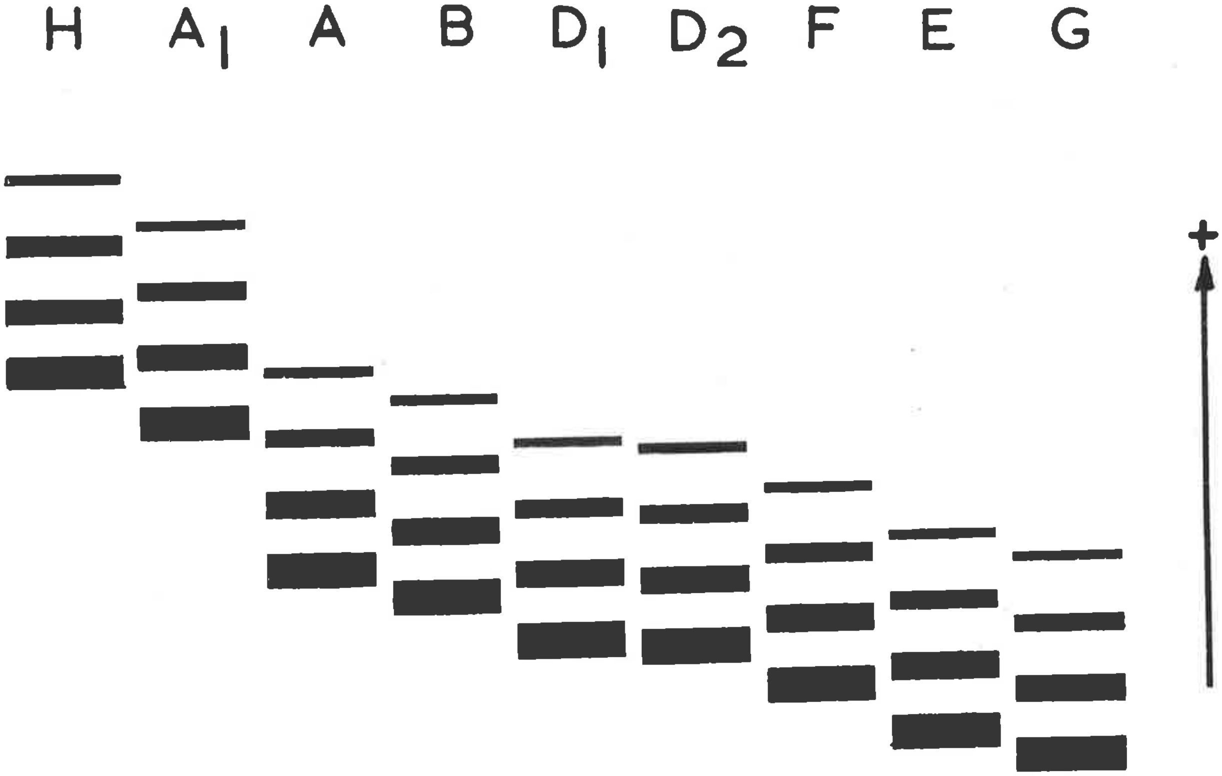
?

In cattle, as in man and in sheep, different breeds and races are characterized by polymorphisms for different transferrin variants and by marked frequency differences for the same variants.

FIGURE 1.

The known transferrin patterns of cattle homozygous for the corresponding alleles at the transferrin locus.

Note: A_1 here is G of Ashton and Lampkin (1965) and is probably more correctly called G_{Kenya}.


H
A1
A
B
D1
D2
F
E
G
+

Thus, transferrins B, F and G (A_1) are confined to zebu breeds whilst A, D_1, D_2 and E are found in both *Bos taurus* and *Bos indicus*. Zebu breeds in general have much higher frequencies of $\underline{Tf}^E$ than temperate breeds although within temperate breeds its frequency varies from very low or absent in Jersey and Guernsey cattle to 0.62% in the British White breed. Jamieson (1966) has documented a large number of transferrin gene frequencies for different cattle breeds.

Some studies have been made on the transferrins of other genera of ruminants. Braend and Stormont (1963) could find no variation in the transferrins of 113 American bison (Bison bison) and noted at that time that the transferrin phenotype was indistinguishable from cattle transferrin A. Later however, using a different gel buffer system, Stormont (1964) showed that the bison transferrin pattern was resolvable into five zones, the slowest of which was slightly faster than the slowest zone of cattle TfA. Other workers (Braend and Gasparski, 1967) demonstrated that the European bison (*Bison bonasus*) had a transferrin pattern indistinguishable from that of *Bison bison* and similar findings have been reported for the African buffalo, *Syncerus caffer* (Osterhoff and Young, 1966). A preliminary report on transferrins in Bulgarian water buffalo cows (possibly *Bubalus*) indicated that the transferrins of these animals showed the zone quartet typical of cattle transferrin but electrophoretic variants were not those observed in European cattle (Makaveev, 1966). These observations supported the conclusions of Datta (1963) and Datta and Stone (1963b) that the serological relation-

2.

ship between the Indian water buffalo (*Bubalus bubalis*) and domestic cattle was not as close as between bison and cattle. The eland (*Taurotragus oryx*) an antelope in a tribe closely related to that of cattle (Figure 2) did not have the cattle transferrin zone quartet but had a zone duo (Ashton and Carr, 1965) and studies on the reindeer (Gahne and Rendel, 1961; Braend, 1964) and the Norwegian elk (Moose) (Braend, 1962) indicated that neither had the cattle transferrin quartet, although the exact relationship between moose and cattle transferrin was not apparent from Braend's figure. The taxonomic relationship between these animals and cattle is remote (Figure 2). Where is Fig 2

A number of observations have been made on the departure from random mating expectations and on disturbed segregation ratios at the transferrin locus in sheep (Khattab et al., 1964; Sartore, 1964; Dassat and Sartore, 1964; Cooper, 1966), and Ashton (1965b) has summarized the evidence for an effect of transferrin type on the reproductive performance of cattle. There is some evidence for mother-foetus incompatibility, superior fitness of heterozygotes and differential fertility of heterozygous and homozygous animals. However, much of the work in this field was carried out before Kristjansson and Hickman (1965) and Ashton (1965a) had described the subdivision of Tf^{D} into Tf^{D1} and Tf^{D2}. Findings relating to heterozygotes (AD, AE, DE) and homozygotes (A, D, E) are therefore to be considered with this in mind, even though the classification of heterozygotes and homozygotes without regard to the subdivision of Tf^{D} is inconsistent only with re-

FIGURE 2

THE CLASSIFICATION OF CATTLE AND RELATED GENERA
(After Simpson, 1945)

ORDER ARTIODACTYLA
SUBORDER RUMINANTIA
INFRAORDER PECORA
SUPERFAMILY CERVOIDEA
FAMILY CERVIDAE (deer, e.g. red deer, reindeer, elk, moose)
SUPERFAMILY BOVOIDEA
FAMILY BOVIDAE
SUBFAMILY BOVINAE
Tribe Strepsicerotini (antelopes, e.g. eland, kudu)
Tribe Bovini
Bubalus bubalis (Asiatic buffalo)
Syncerus caffer (African buffalo)
Bison bonasus (European bison; Wisent)
⌿ " bison (American bison)
* Bibos gaurus (Gaur)
" frontalis (Gayal)
" banteng (Banteng)
Bos primigenius (extinct Auroch)
" indicus (Zebu cattle)
" taurus (European cattle)
" grunniens (Tibetan Yak)

⌿ Roe (1951) regards the American bison as *Bos americanus*.
Deakin *et al*. (1942) use the name *Bison americanus*.
Simpson (1945) and Walker (1964) both classify the American bison as *Bison bison*.

* Walker (1964) classifies these as: *Bos gaurus*
Bos frontalis
Bos banteng

*, ⌿ Zeuner (1963) regards *Bison* and *Bibos* as subgenera of *Bos*.

gard to the class TfD_1D_2 which is drawn from the homozygous class to enter the heterozygous class. Any observations on the different performance of the two classes could therefore be an underestimate rather than an overestimate of any true performance difference.

(i) Dam-foetus incompatibility

Ashton (1959b) found that in Friesian and Ayrshire cattle, reciprocal matings involving TfA or TfD by TfAD gave an excess of offspring with transferrin type like their dam. He proposed that the underlying mechanism for this was maternal-foetal incompatibility. If one considers his data for Friesian cattle (Table 2) then although

TABLE 2

MATERNAL-FOETAL INCOMPATIBILITY DATA OF ASHTON (1959b)

MATING TYPE	PARENTS		OFFSPRING		χ^2_1	P
	FEMALE	MALE	LIKE-DAM	LIKE-SIRE		
1	A	AD	19	11	2.13	.1 - .2
2	AD	A	13	8		
3	D	AD	17	9		
4	AD	D	32	25		
TOTAL			81	53	5.85	.01 - .02

the data are homogeneous ($\chi^2_3 = 0.48$) and on statistical grounds may be combined as was done by the author, there are other bases for not combining the results of all mating types. Mating types 2 and 4 cannot produce offspring with a transferrin not found in the dam and therefore it is difficult to imagine a priori how these matings could produce foetuses incompatible with their dam. Thus, probably as a result of similar reasoning to this, Ashton (1965b) used only mating types 1 and 3 in incompatibility studies and still obtained a significant excess of like-dam offspring in these two mating types combined (478:409, $\chi^2_1 = 5.36$, $P < 0.05$). However, when one considers the subdivision of transferrin D into D_1, D_2 and D_1D_2, mating type 3 cannot be regarded as suitable for the examination of data for maternal foetal incompatibility, since if the D allele in each parent is a different one ($\underline{Tf}^{D1}$ and $\underline{Tf}^{D2}$), a simple like-dam, like-sire classification is not possible. Only data from mating type 1 are suitable for maternal-foetal incompatibility studies when transferrin D is not subdivided. Data on mating type 1 collated from a number of sources by Ashton (1965b), show no evidence of maternal-foetal incompatibility (268:241, $\chi^2_1 = 1.43$, $.2 < P < .3$, writer's calculation) and neither does that in Table 2. Ashton (1959b) also found that in similar matings involving transferrin E an excess of offspring carrying the $\underline{Tf}^{E}$ allele was found where the sire carried $\underline{Tf}^{E}$. Otherwise offspring carrying $\underline{Tf}^{E}$ were found in lower frequency than expected. Similar findings to those discussed above have been described by Buschmann (1963). Studies on aberrant segregation in different populations by Ashton (1961), Gahne (1961), Ashton and Fallon (1962), Brummerstedt-Hanssen et al. (1963), Rausch et al. (1963), Datta et al. (1965) and

Jamieson (1965) failed to confirm these observations and Moustgaard *et al.* (1960) and Hines (1965) could find no immunological differences between transferrin types. If mother-offspring incompatibility was a real effect of the transferrin locus, then matings between like homozygotes would be at a selective advantage. As pointed out by Hickman and Dunn (1961), if this were so it could lead to fixation of the allele with the highest frequency at the outset, a trend for which there is no evidence at present in cattle.

(ii) Superior fitness of heterozygotes

Ashton and Fallon (1962) examined "returns-to-service" data from matings involving like-homozygotes (producing homozygotes *in utero*) and unlike-homozygotes (producing heterozygotes *in utero*). They found that whereas there was no difference between the efficiency of the two types of mating when "short-returns" (0-24 days) were considered, there was a significant excess of cows in the mating class "like homozygotes" that returned to service 25 days or more after insemination. This was interpreted as indicating a superior fitness of heterozygotes *in utero*. Work in New Zealand (quoted in New Zealand D.P.M.B., 1963) showed similar though non-significant trends. These observations would seem to contradict those made on maternal-foetal incompatibility since matings involving like homozygotes cannot produce offspring incompatible with the dams, whereas mating involving unlike homozygotes all produce incompatible offspring. Thus one might expect that matings involving like homozygotes would be more successful than those involving unlike homozygotes if maternal-foetal incompatibility was having a significant effect on foetal mortality, whereas the opposite was observed. With the subdivision of $\underline{Tf}^{D}$ into $\underline{Tf}^{D_1}$ and $\underline{Tf}^{D_2}$, matings between like homozygotes of the type D x D are actually composed of the types D_1 x D_1, D_2 x D_2 (like homozygotes), D_1 x D_2 (unlike homozygotes) and D_1 x D_1D_2, D_2 x D_1D_2, D_1D_2 x D_1D_2 (homozygotes x heterozygotes and heterozygotes x heterozygotes) where 14% of matings are of the first type, 14% of the second type and 72% of the third type when $\underline{Tf}^{D_1}$ and $\underline{Tf}^{D_2}$ have equal frequency. Thus, only 14% of the D x D matings are actually like homozygotes in this case. Similarly, a large proportion of the matings between unlike homozygotes also involve 50% heterozygotes. The observations are therefore very difficult to interpret in terms of heterozygote superiority _in utero_ but could be re-evaluated in terms of A x A and A x "not-A".

Further evidence for heterozygote superiority has been put forward by Ashton (1965b) using data from a number of sources. Matings of the type AD x AD, A x AD, D x AD which would be expected to give equal numbers of heterozygotes and homozygotes among the progeny and which would not involve incompatible offspring, were found to give a significant excess of heterozygotes. Datta _et al._ (1965) could not confirm this observation although they cited other evidence to support it (Gahne, personal communication). Again, interpretation in the same sense is not now possible since $\underline{Tf}^{D}$ is actually two alleles.

(iii) Differential fertility of heterozygotes and homozygotes

Ashton (1961) found that matings between homozygotes (like or unlike) were significantly more successful than those involving at least one heterozygote. Similar findings were reported by Ogden (1961). The high proportion of heterozygotes (D_1D_2) now known to exist among homozygotes makes interpretation of this difficult.

Irrespective of the transferrin type of bulls, Ashton (1961) found that homozygous cows in one region of Queensland had higher "breeding efficiency" than heterozygous cows, "breeding efficiency" being measured by the proportion of cows not returning to service. In another region, irrespective of the cow's transferrin type, homozygous bulls had a higher "breeding efficiency" than heterozygous bulls, where a bull's breeding efficiency is determined by the proportion of cows to which it is mated that do not return to service. Ashton (1965b) observed that in pooled data homozygous bulls were consistently more fertile than heterozygous bulls although the significance of this was not tested. Also, the distribution of heterozygous and homozygous bulls selected for artificial breeding was significantly biased towards homozygotes, and Ashton (1965b) suggested that this may have been a result of the increased fertility of bulls homozygous for alleles at the transferrin locus.

(iv) Departures from frequencies expected with random mating

For a genetic polymorphism involving two alleles, the most likely situation leading to a selectively balanced polymorphism is the superior fitness of the heterozygote (Fisher, 1930). For more than two alleles a number of different systems or sets of selective value can give rise to a selectively balanced polymorphism (Owen, 1954; Li, 1955; Kimura, 1956; Tallis, 1966). Thus the simple model assigning a relative viability of 1.00 for transferrin AD, 0.85 for A and 0.85 for D (Ashton, 1965b), whilst accounting for some of the observed effects of the transferrin locus on breeding efficiency and departure from random mating expectation for two transferrin alleles, does not consider all the selective forces which may be operating to maintain a polymorphism involving three, four, five or six alleles. However, regardless of the exact nature of the forces maintaining the polymorphism, heterozygote advantage could lead to an excess of heterozygotes over that expected. Ashton and Fallon (1962) reported a significant 6% excess of heterozygotes in one population but three other populations and one reported by Gahne, et al (1960) had an excess of heterozygotes which was not significant. Other workers (Braend et al., 1962; Gahne, 1961; Datta et al., 1965) did not confirm this finding although extensive data published by Jamieson (1965) showed significant deviation in favour of heterozygotes or homozygotes depending upon the herd examined. Ashton (1965b) pooled data from Ashton and Lampkin (1965b) and Ashton et al.

(1966) and obtained a significant excess of transferrin heterozygotes. These results were obtained from data in which $\underline{Tf}^{D}$ was subdivided into $\underline{Tf}^{D1}$ and $\underline{Tf}^{D2}$ as were those of Jamieson (1965).

From the foregoing discussion on the results of workers studying the effect of transferrin type on viability and fertility it is apparent that the literature contains numerous examples of observations which have not been confirmed by other workers. It is unlikely that all significant effects are chance observations and it is possible that both the genetic and physical environment of the animals could influence the effect of alleles at the transferrin locus on viability and fertility. Variation in the frequency of $\underline{Tf}^{D1}$ and $\underline{Tf}^{D2}$ may also be a factor contributing to the inconsistencies in results obtained by different workers if any of the real effects are attributable to one D allele or the other.

b. The J blood group of cattle

The J blood group was first described in cattle by Ferguson *et al.* (1942) when they observed that the sera of some individuals had a naturally occurring antibody which lysed the red cells of other individuals in the presence of rabbit complement. It was subsequently found that the J antigen was primarily a serum constituent which became adsorbed onto the red cells sometime after birth (Stormont, 1949) and since twins showing erythrocyte mocaicism (when tested with other reagents) were found to have distinctly different J blood groups it was concluded that J substance was not produced by the same tissues responsible for erythropoiesis. Stone and Irwin (1954) classified cattle into three groups: (1) J^{CS}, those with the J antigen on their erythrocytes and in their serum, (2) J^{S}, those with J antigen in their serum only, and (3) j^{a}, those without the J antigen. The last group had natural anti-J in their sera at titres depending upon the season of the year (Stone, 1956). A more detailed review is given by Stone (1962). Conneally et al. (1960) found that the frequency distributions of the J^{S} and J^{CS} groups each showed four modes and they suggested a minimum of four alleles controlling antigen titre in each group.

It has been shown (Stormont, 1951) that natural bovine anti-J has a much greater avidity for sheep R cells than for strongly reacting J red cells. Sheep anti-R however, would not lyse cattle J red cells. Thus, the R and J blood groups of sheep and cattle respectively are related serologically and also in the sense that they are both primarily soluble specific blood group substances (SSS) of the serum. The A antigen of man is also serologically related to both the R and J antigens and to the A antigen of swine (Stormont, 1949; Sprague, 1955; Neimann-Sorensen et al., 1954) but human A substance is different from the others in that it forms an integral part of the red cell wall in the blood at least.

Stormont (1951) working on the R blood group of sheep (see Appendix II) found a naturally occurring antibody in the serum of some cattle which would react with R-negative sheep red cells (O) but not with R-positive sheep red cells(R). Using this antibody (natural bovine anti-sheep O) he demonstrated that the R/O difference was controlled by two allelic genes *R* and *r*, with the O phenotype recessive to the R. Rendel et al. (1954) found a third class of sheep (i) whose cells reacted with neither antibody. The observation that both R and O sheep had O substance in their saliva led Rendel (1957) to propose that the O substance was a product of another gene, *I*, at a different locus. Thus, the R-O-i blood group system in sheep appears to be controlled by two alleles at each of two loci. Animals of genotype *I-R-* are blood group R, those of genotype *I-rr* are blood group O and those of genotype *ii--* are blood group i (see Appendix II).

Studies on the distribution and inheritance of natural bovine anti-sheep O in cattle indicated that the production of the antibody was under the control of a single dominant gene (Sprague, 1958b). Using this antibody for inhibition tests Sprague (1958a) demonstrated the presence of an O substance in some cattle sera (both J positive and J negative) although the red cells of these animals were not lysed with the reagent. The O substance of cattle was designated "Oc" and studies on its inheritance indicate that observations on family and population data were best interpreted as the result of the segregation of four alleles at the J locus. That is J^{J}, j, J^{JOc}, and J^{Oc}. The proposed phenotype-genotype relationships in the J-Oc system are given in Table 3. There is a rather curious relationship between the proposed alleles at the J locus since in the presence of the j allele neither J^{JOc} nor J^{Oc} produce any Oc substance. That is J^{JOc}/j individuals

Indee

TABLE 3

PROPOSED PHENOTYPE - GENOTYPE RELATIONSHIPS IN THE JOc BLOOD-GROUP SYSTEM (FROM SPRAGUE, 1958a)

Phenotype			Possible Genotypes
Notation	Serum Inhibits	Blood Group Substance in Serum	
JOc	anti-O anti-J	J and Oc	$\underline{J}^{JOc}/\underline{J}^{JOc}$, $\underline{J}^{JOc}/\underline{J}^{Oc}$
J	anti-J	J	$\underline{J}^{J}/\underline{J}^{J}$, $\underline{J}^{J}/\underline{J}^{Oc}$, $\underline{J}^{J}/\underline{j}$, $\underline{J}^{J}/\underline{J}^{JOc}$, $\underline{J}^{JOc}/\underline{j}$
Oc	anti-O	Oc	$\underline{J}^{Oc}/\underline{J}^{Oc}$
"-"	neither antibody	neither antigen	$\underline{J}^{Oc}/\underline{j}$, $\underline{j}/\underline{j}$

have the J phenotype and $\underline{J}^{Oc}/\underline{j}$ individuals have the "–" phenotype. This point is taken up later in the discussion (p. 74).

Since the titre of Oc substance in the serum of cattle is variable (Rendel and Suzuki, 1964) it is likely that the JOc and Oc phenotypes may be subdivided like $\underline{J}^{CS}$ and $\underline{J}^{S}$ although relevance of the finding of four modes in the $\underline{J}^{CS}$ and $\underline{J}^{S}$ classifications (Conneally *et al.*, 1960) to the J, JOc, Oc, and "–" classifications is not clear. There is a negative correlation between the amount of Oc substance and the amount of J substance in sera (Rendel and Suzuki, 1964) and these workers suggested that both antigens may have a common precursor. In this connexion it is interesting to note that the Oc substance of cattle is serologically related to the H substance of man (as well as to the O substance of sheep and swine) and the A and H substances of man are believed to originate from a common precursor substance (Race and Sanger, 1962).

It is!

Extensive data on the distribution of J alleles in different breeds are not available. However, Stormont *et al.* (1961) have found a very similar system in the American buffalo (*Bison bison*) where the inhibition types J, JOc and Oc are described. The European bison (Wisent; *Bison bonasus*) and the Indian water buffalo (*Bubalus bubalis*) also have a J blood group system (Gasparski and Dubiski, 1962; Gasparski, 1964; Datta and Stone, 1963b). Studies on the Norwegian elk (Moose; *Alces alces*) by Braend (1962) using a single J reagent revealed a J blood group system similar to, if not identical with, that of cattle. This high degree of similarity between the J blood groups of these widely separated genera is of interest since moose transferrin also shows some similarities to cattle transferrin (Braend, 1962).

Testing of goat sera with sheep and cattle reagents has indicated that goats have B, C and M blood group systems like those of sheep. However, they have a J blood group system like that of cattle, not the R-O-i system of sheep (Stormont and Suzuki, 1961). The blood group system in goats consists of the three inhibition types; JOg, Og and "-" (Suzuki and Stormont, 1961, 1962). The naturally occurring antibodies in the sera of "-" animals are, however, a curious mixture of those found in both cattle and sheep, viz. anti-sheep R and anti-sheep O (Suzuki and Stormont, 1961; Bailey, unpublished).

c. Serum alkaline phosphatases in animals

Electrophoretic variants of the enzyme alkaline phosphatase have been found in the serum of man (Arfors et al., 1963), cattle Gahne, 1963b), sheep (Rendel and Stormont, 1964), goats (Bailey unpublished), chickens (Wilcox, 1963; 1966; Law and Munroe, 1965), and several other species of birds (Beckman and Nilson, 1965). In man, cattle, sheep and goats the polymorphism is different from other serum protein polymorphisms in that the variants differ by the presence or absence of an additional zone of phosphatase activity, the additional zone having variable staining intensity between individuals possessing it. For this reason it has been difficult to carry out studies on the inheritance of the variant types and therefore with the possible exception of cattle (Gahne, 1963b) no formal genetic hypothesis covers all observations.

In man, Arfors _et al._ (1964), Beckman (1964), Bamford _et al._ (1965), Shreffler (1965) and Hope (1966a) have shown that the presence or absence of the B phosphatase zone is related to the presence or absence of the B and H antigens on the red cell and to the secretor status of the individual. Thus, almost all people with B zone activity are ABO blood group B or O and secrete these substances into their saliva. It was concluded by Beckman (1964) that the presence of the B phosphatase zone was dependent upon at least one other factor besides the ABO and secretor genes.

In sheep, Rendel and Stormont (1964), Rendel _et al._ (1964), Rasmusen (1965) and Hope (1966b) showed that the presence of B zone activity in the serum was highly correlated with the presence of the O blood-group substance of the R-O-i system in the serum and on the red cells. This association was not complete since there were some animals of blood group R with B zone activity and some of group O without it; however, Rendel _et al._ (1964) were able to induce B zone activity in a B negative sheep by intravenous injections of saliva containing O blood-group substance.

In cattle no pattern similar to that of man and sheep was observed and a variety of classifications was possible (Gahne, 1963b). Cattle having the A zone were classified as A and those lacking it as O. Gahne interpreted his mating data as indicating that phosphatase patterns were the result of the segregation of two alleles $\underline{F}^{A}$ and $\underline{F}^{O}$ at the phosphatase locus. Thus individuals of genotype $\underline{F}^A/\underline{F}^A$ or $\underline{F}^A/\underline{F}^O$ have phosphatase pattern A and those of genotype $\underline{F}^O/\underline{F}^O$ have phosphatase pattern O. In 128 matings of the type O x O, no A offspring was observed. Rendel and Gahne (1963) examined the association between the J blood group types J^{CS}, J^S and j^a and phosphatase type and found a small but significant excess of animals with A zone activity in the class j^a. Also, in both J^{CS} and J^S classifications the titre of J substance was higher in type O phosphatase cows that in type A cows. This observation was repeated by Rendel and Suzuki (1964) who also showed that there was a tendency for animals with Oc substance to have the O phosphatase phenotype. The failure to find a close association between the presence and absence of a serum phosphatase isozyme and the presence and absence of a blood group substance in cattle is probably a reflection of the close adherence of alkaline phosphatase inheritance to formal Mendelian theory in that species (p.23). That is not to say that there is no phosphatase isozyme associated with the inheritance of an H-like blood group substance in cattle as there is in sheep and man (p.23).

d. Blood group substances and transferrins in foetal blood

1. Red-cell and serum antigens

In man, the A and B antigens were detectable on the red cells of foetuses at 37 days (Kemp, 1930). However, the antigen specificity was not that found in adults since A_1 and A_2 were not distinguishable at this stage. Work on Lewis blood groups indicated that foetuses did not have Lewis a or b substances on their red cells (Andresen, 1948; Jordal, 1956), however, Lawler and Marshall (1961) demonstrated the presence of Lewis substance in the serum and saliva of newborn infants so in this respect the development of the Lewis groups is similar to the development of the R and O blood groups of sheep (Ycas, 1949; Rendel, 1957) and the J^{CS} blood group of cattle (Stormont, 1949) where the antigens at birth are detectable only in the serum but become adsorbed onto the red cells within the first month of life. The soluble blood group substance Gc in man has received some attention with respect to its time of development. It was certainly detectable at the time of birth (Cleve and Bearn, 1962; Hess and Butler, 1962; Hirschfeld, 1962; Hirschfeld and Lunnel, 1962; Nerstrøm, 1963) but Hirschfeld and Lunnel (1962) and Cleve and Bearn (1962) could not detect it in foetuses as young as 17 weeks. The time of appearance fell in the range 20-25 weeks; however, more recently Melartin _et al_. (1966) demonstrated foetal Gc groups as early as 9 weeks.

In cattle foetuses Schmid and Buschmann (1962) showed that the red cell antigens were well-developed in a foetus 17 mm long (4-5 weeks old): however, they found evidence that the red cell antigens were not as strongly developed as in adults. The J substance concentration in sera was said to be associated with foetal age; however, if foetuses show a genetically determined range of titres as do adults then it seems unlikely that the observations on the five foetuses examined represent the full range of variability. Examination of 113 foetal sera revealed no natural anti-J activity indicating that this antibody

Good point

at least is not transferred across the placental barrier.

2. Transferrin

Parker and Bearn (1962) and Parker *et al.* (1963) found transferrin in the serum of human foetuses but observed that in addition to the major component found in adults, there were four trailing minor bands. These were considered to be due to the addition of different amounts of carbohydrate to the transferrin molecule such that the slowest (fourth) minor component had no carbohydrate moiety attached, the third had one, the second had two, the first had three and the major band had four (Jamieson, 1964). Melartin *et al.* (1966) found that human foetuses could have transferrin different from that of their mother (presumably inherited from the father) and that the transferrin concentration in foetal serum was greatly reduced in young foetuses.

Similar increases in transferrin concentration with foetal age have been noted in the sheep (Bailey, unpublished), the mouse (Pantelouris and Hale, 1962) and the rat (Wise *et al.*, 1963). In the latter species Wise *et al.* (1963) found that the fast minor transferrin zones of foetuses had a relatively higher concentration than in adults. If the slow trailing minor bands of foetal transferrin in man are due to a low concentration of an enzyme attaching negatively charged carbohydrate to transferrin (adult transferrin having a full complement of carbohydrate), and if the normal form of the rat transferrin major band is saturated with carbohydrate, then this carbohydrate moiety would have to be positively charged for its removal to produce minor bands with mobility faster than the major band.

Foetal cattle transferrins were studied by Buschmann and Schmid (1961) and Schmid and Buschmann (1962) who reported that they found all the adult transferrin types except transferrin E (they did not subdivide $\underline{Tf}^{D}$ into $\underline{Tf}^{D_1}$ and $\underline{Tf}^{D_2}$).

If the information on the structure of human transferrin yielded by a study of foetal transferrin patterns is taken as a precedent for the type of information available from such studies, then a more detailed study of cattle foetal transferrin could be of value.

II. MATERIALS AND METHODS

a. Collection of blood samples

Blood was collected from cattle using a modification of the method described by Brown and Carrow (1963). Using a 5 ml sterile disposable syringe and a 1" x 18 hypodermic needle, blood was obtained from the coccygeal blood vessels by inserting the needle into the ventral surface of the tail in the hairless area about 4" from the anus. The blood was then either allowed to clot in the syringe and transferred to a dry, sterile bottle or was emptied immediately into citrated saline (5 g NaCl, 20 g sodium citrate/1000 ml) if red cells were required. Tail sampling was well suited for the fast bleeding of large numbers of animals; however, for large volumes of blood from individual animals jugular bleeding was used.

Blood was obtained from slaughtered abattoirs material by opening the heart of an animal immediately after the heart and lungs were removed. Up to 500 ml could be obtained in this way if required. Foetuses were removed from the uterus and bled from the jugular vein if large enough, i.e. with hair, or dissected open and bled from the large veins of the chest cavity. It was found that unless foetal serum was taken off within 12 hours of collection, haemolysis at 5°C was appreciable. The fragility of foetal red cells also made centrifugation undesirable. It should be pointed out here that blood obtained from abattoirs material often failed to clot, in which case the plasma invariably clotted after being drawn off and stored.

All serum was stored at -25°C.

b. Sources of blood samples

1. Stud herds

Eight purebred Friesian and 24 purebred Jersey herds were selected for blood sampling. Details of these are given in Section B, page 95 and Table 31.

2. Northfield research dairy

The South Australian Agriculture Department's experimental dairy herd of mixed breed was made available for blood sampling and was used extensively for the collection of standard sera and red cells.

3. Artificial breeding centre

Artificial breeding is used exclusively at the Northfield dairy so that the bleeding of all bulls provided a convenient source of family data.

4. Abattoirs material

The Metropolitan and Export Abattoirs Board co-operated fully in the collection of incomplete family data in the form of dam-foetus pairs. Approximately 30 pregnant cows could be obtained in four hours by a single operator although this represented only half of those pregnant animals passing through the abattoirs. Foetuses _in utero_ were tagged as they fell from the cow and no pair was bled unless the author actually saw the foetus removed.

Good

c. Starch gel electrophoresis procedures

1. Technical aspects

The electrophoresis procedure was basically that described by Smithies (1959) with some modifications.

(i) High voltage electrophoresis was facilitated by the use of a brass cooling tank coated with a heat conducting, electrical insulator, Melanex. The apparatus was supplied by Paton Industries, Beaumont, South Australia, and consisted of a brass tank 16 cm x 31 cm with P.V.C. sides attached to give a bed thickness of 5 mm (Figures 3 and 25, p.131).

(ii) Gels were not prepared as originally described by Smithies (1955) but by using the method described by Kristjansson (1963) where an aliquot of cold buffer was mixed with the starch and the remainder boiled and added to the starch slurry. The proportion of buffer used to make the slurry depended upon the total volume of gel prepared and upon the batch of starch being used. Table 4 gives the

TABLE 4

PREPARATION OF STARCH GEL USING THE METHOD OF KRISTJANSSON (1963)

Starch* lot	Manufacturer's recommended concentration	Concentration used†	Volume slurry at 25°C	
			500 ml gel	1800 ml gel
229-1	10.3	14.5	110	450
248-1	9.8	13.5	130	600
249-1	9.6	13.5	130	600

* Connaught Medical Research Laboratories, Toronto, Canada.

† Discontinuous buffer systems only. For continuous systems, used as recommended.

FIGURE 3.

Four starch gels set up in parallel showing:-

A. Double cathodic electrolyte trays.

B. Foam plastic bridges.

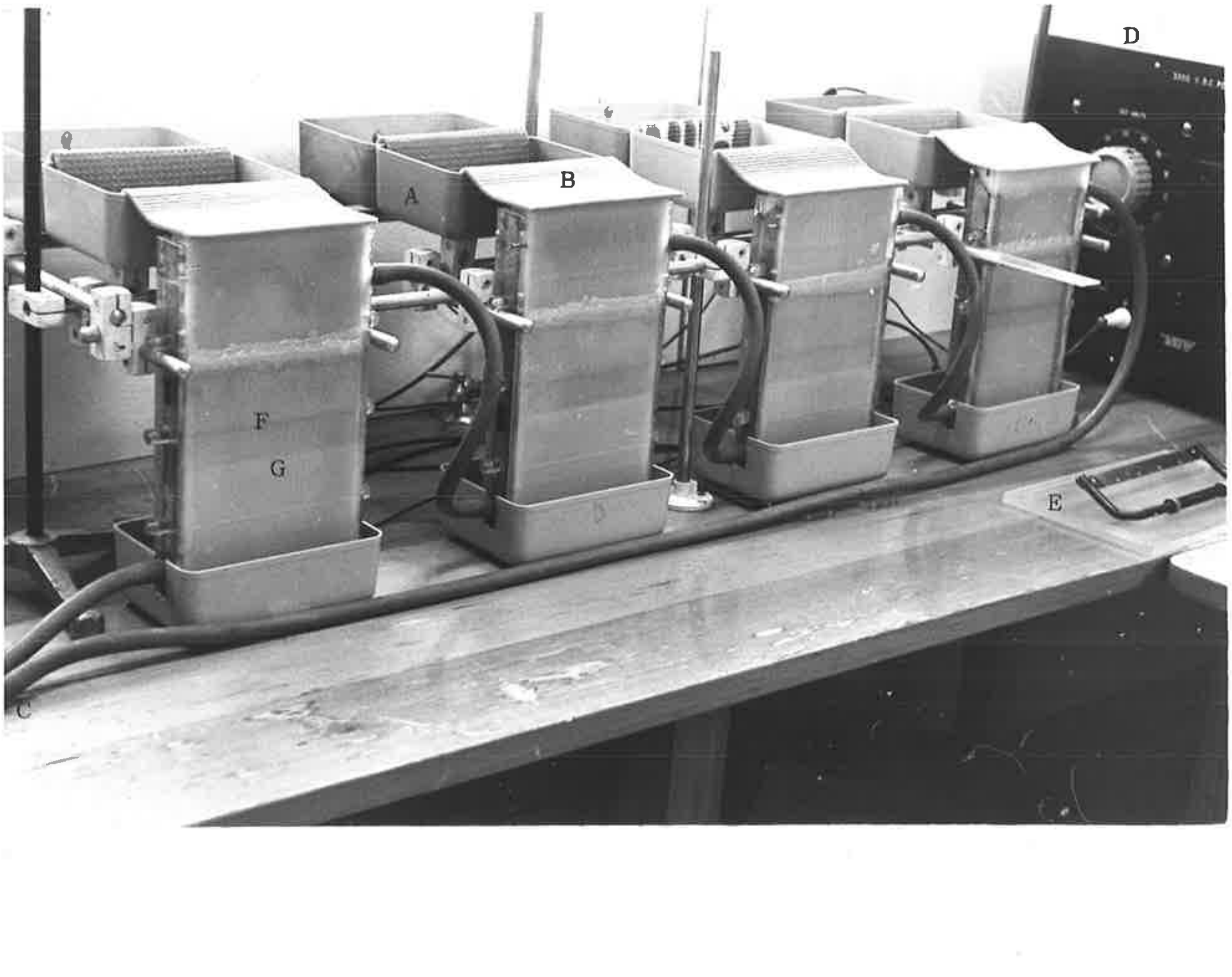
C. Tanks cooled in series using tap water.

D. Power pack.

E. Cutting tray and knife.

F. Albumin bands.

G. Migrating boundary.


A
B
C
D
E
F
G

volumes of buffer used for three starch batches and three volumes of gel. After pouring, gels were run within two hours.

(iii) Serum samples were inserted into sample slots which were moulded into the gel at the time of pouring. The standard slot formers were of 'Teflon' or laminated bakerlite which produced 12 slots each capable of holding 0.04 ml serum. Since this quantity of serum was generally in excess of that required to give clear transferrin patterns with adult sera, the serum was diluted 1/3. With the use of high starch concentration however, it was possible to use a slot former made from a stainless steel skin grafting knife blade which held only 0.01 ml serum. This made tedious dilution of each sample unnecessary.

2. Buffer systems

The following buffer systems were used for the electrophoresis of cattle sera. Section d. of this chapter (p.35) gives details as to where each was applied.

Ferguson and Wallace (1961) described a modification of Poulik's (1957) discontinuous buffer system which they used to characterize pituitary proteins. The system was later used by Ashton and Ferguson (1963) for the electrophoresis of sheep serum and the typing of sheep transferrins.

Gahne (1963a) modified the above tris-citrate/lithium borate system for use with cattle serum. This system had two major advantages over the Ferguson and Wallace (1961) system for such sera. Resolution of transferrins was better and since haemoglobin migrated faster than transferrin in Gahne's (1963a) system any haemolysis of red cells did not interfere with transferrin typing as it did in the first system described (Fig. 4).

Ashton (1965) described a system similar to Gahne's (1963a) system which he used to subdivide cattle transferrin D into D_1 and D_2. In the author's experience and in Ashton's own opinion (Kristjansson and Ashton, 1966) this system did not give resolution comparable with that developed concurrently by Kristjansson and Hickman (1965) although resolution of B and F variants was better.

Kristjansson and Hickman (1965) developed a tris-cacodylate/sodium borate system for the resolution of D_1 and D_2 phenotypes. This system employed a lower pH (7.5) and higher starch concentration than the above systems and gave resolution not previously achieved. A similar system at pH 7.3 described recently by Makarechian and Howell (1967) gave extremely good resolution of the D_1D_2 phenotype making classification of this phenotype very simple.

This study has shown that the use of Gahne's (1963a) lithium borate electrolyte in place of Smithies' (1955) sodium borate electrolyte gave improved resolution probably due to the lower running temperature and more intense protein staining obtained (Fig. 5).

3. Dye markers in starch gel electrophoresis

The use of tincture of merthiolate as a preservative in large preparations of gel buffers by Dr. D.W. Cooper of this laboratory proved to be of value also as a marker of albumin during electrophoresis.

FIGURE 4.

Cattle standard transferrin types as resolved in Gahne's (1963a) tris-citrate/lithium-borate buffer system. Haemoglobin, in any haemolysed samples, migrates between transferrin and serum albumin.

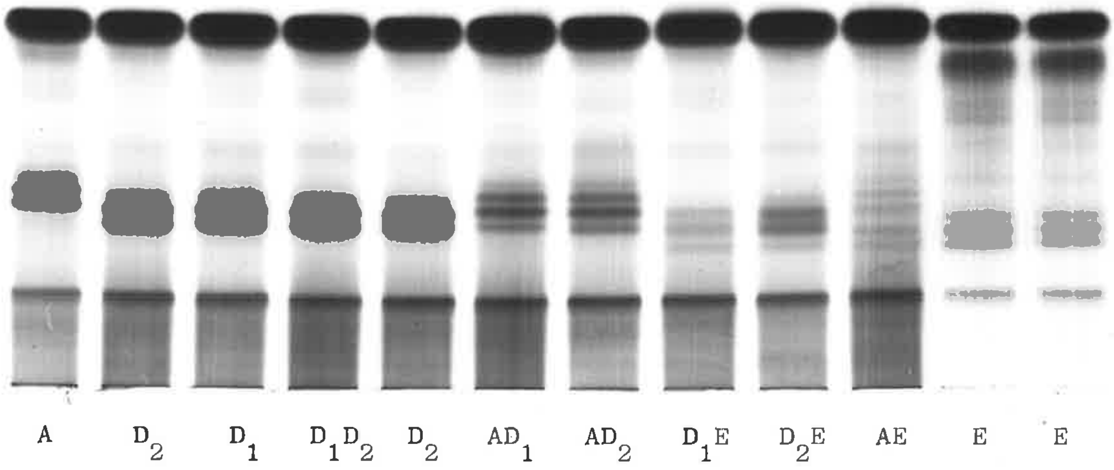
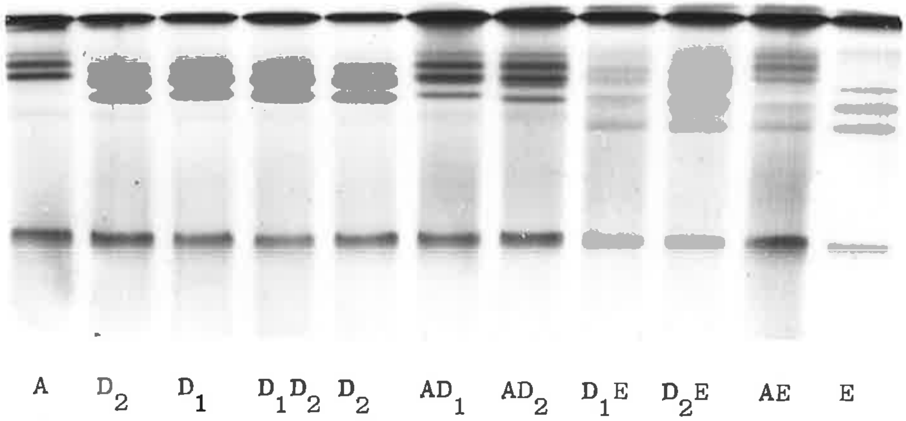

A
D_2
D_1
D_1D_2
D_2
AD_1
AD_2
D_1E
D_2E
AE
E
E

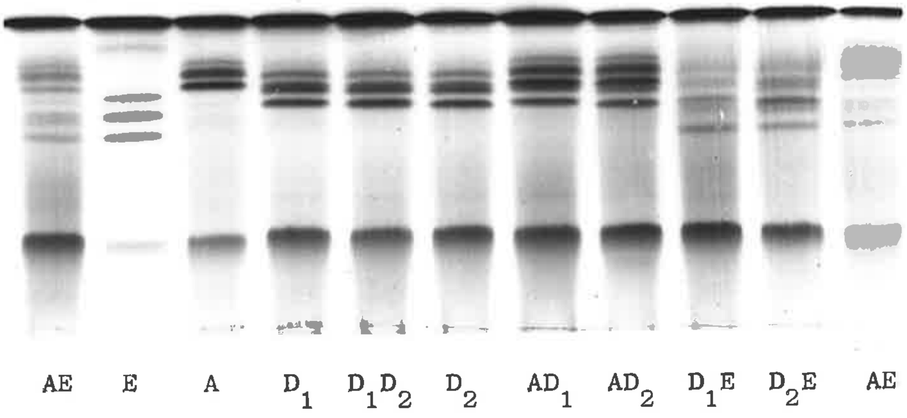
FIGURE 5.

Cattle transferrin standards after electrophoresis of sera in the tris-cacodylate/lithium-borate system described in this thesis. Haemoglobin in this system migrates between transferrin and the slow α_2 protein zone. Modified Kristjansson Hickman, 1965

TOP Running temperature 25°C.

BOTTOM Running temperature 5°C. Note the resolution of D_1 and D_2 into five zones at low temperature.


A
D_2
D_1
D_1D_2
D_2
AD_1
AD_2
D_1E
D_2E
AE
E


AE
E
A
D_1
D_1D_2
D_2
AD_1
AD_2
D_1E
D_2E
AE

The dye used in the tincture, which was probably eosin since its effect could be duplicated using eosin, became attached to the trailing edge of the albumin during electrophoresis. The migrating boundary of dis continuous buffer systems was also more easily observed. This chance observation suggested the possibility of using other dyes as an aid to observing proteins during electrophoresis. Subsequently 26 indicators and dyes were screened in an attempt to find a better marker than eosin and the following were found suitable:-

Bromophenol blue
Bromothymol blue
Bromcresol green
Trypan blue
Indigo carmine
Orcein
Carmine
Orange G
Acid Fuchsin

Outstanding among these was bromophenol blue which was then adopted generally as a dyemarker and added to all gel buffers in concentration at which a blue colour was just observable. Bromophenol blue attached to the trailing edge of the albumin zone and was swept out of the gel by the migrating boundary in discontinuous systems. When the front passed through the albumin all dye associated with the front was transferred to the albumin, making it clearly observable and enabling accurate measurements to be made. The use of bromophenol blue as a dyemarker has also been described by Bloemendahl (1963).

4. Staining procedures

Sliced gels were stained for protein using a concentrated amido black solution (3.7 g amido black, 280 ml methanol, 250 ml water, 50 ml acetic acid) and staining time of 30 seconds, after which the stain was removed by washing in a 5:5:1 methanol:water:acetic acid mixture.

Alkaline phosphatase activity was detected in gels using a modification of Gahne's (1963b) receipe, (Gahne; personal communication). That is,

100 ml 0.1 M Tris-HCl pH 8.6 containing 0.01 M $MgCl_2$
50 mg Na-α-naphthyl phosphate
50 mg Fast Garnet G.B.C. salt
Incubated 2 hrs. at 24-30°C.

5. Autoradiography

The autoradiographic technique for demonstrating the iron-binding capacity of transferrins was similar to that described by Cooper and Sharman (1964) with some modifications to improve resolution. 0.6 μc. $Fe^{59}Cl_3$ in 1 drop of 0.1 N HCl were dried onto the inside of a small tube at 40°C. The tube was cooled and 2 drops of serum were added to the tube and incubated 15 minutes at 25°C before placing in the gel slots. After electrophoresis gels were sliced, placed between two plastic sheets and put, cut surface upwards, into a photographic-paper box 10" x 8".

All air bubbles were smoothed out from between the plastic and the gel was then tightly taped to the bottom of the box to prevent curling during freezing. The box (without lid) was placed in the bottom of a deep freeze for two hours at -30°C after which time the gel was hard and brittle. In the dark room the gel was removed from the box, an X-ray plate taped in its place, the plastic sheet was removed from the cut surface of the gel and the frozen gel placed directly into contact with the X-ray plate. The gel was then taped securely to the plate using the plastic sheet on the back of the gel to provide adhesion and the lid was placed on the box which was wrapped in aluminium foil and returned to the bottom of the deep freeze for three days. An autoradiograph is shown in Figure 7 (p. 48).

6. Photography

Gel and autoradiographs were photographed dry on a white tile in reflected light using Leitz copying equipment and a 35 mm Ilford "Micro-Neg Pan" film.

d. Transferrin typing

Why not Gahne 63 page 31

For the classification of dairy cattle according to transferrin type in Section B of this thesis, the buffer system described by Ferguson and Wallace (1961) was used. For this typing 0.04 ml serum was placed into slots 9.5 cm from the cathodic end of the gel and electrophoresis was stopped when the migrating boundary had moved 14 cm past the slots. Transferrins were classified without regard to the subdivision of D into D_1 and D_2.

Unclear!

2

Gahne's (1963a) system was used for the initial electrophoresis of all other sera. 0.04 ml serum was loaded into slots placed 10.2 cm from the cathode. Since this distance included a starch block 7 mm thick, 3 cm long and 16 cm wide (33.6 cc) then the effective distance from the cathode was 9.5 cm (distance from slots to block) + 4.2 cm (equivalent length of starch 16 cm wide and 5 mm thick) = 13.7 cm. Electrophoresis was continued at 16 volts/cm until the trailing edge of the albumin zone had migrated 6 cm from the origin. This usually took 4 hours. Using this system sera were classified as transferrin A, AD, D, AE, D_1E or D_2E. It was considered easier to carry out this initial classification using Gahne's (1963a) system first since subdivision of D into D_1 and D_2 required close approximation of a sample to a standard, a requirement not satisfied if there were TfA samples on a gel. When it was found that AD_1 and AD_2 could be distinguished qualitatively without comparison of the mobility of the fourth D zone initial typing in Gahne's (1963a) system was abandoned, however all sera typed as transferrin D were rerun together to distinguish D_1, D_2 and D_1D_2 reliably.

To subdivide D into D_1 and D_2 the system of Kristjansson and Hickman (1965) was used with the modification in electrolyte composition as described above. 0.01 ml serum was loaded into slots placed 7.2 cm from the cathode (effective distance = 6.5 + 4.2 = 10.7 cm) and electrophoresis was continued at 16 volts/cm until the blue boundary, which did not make any gain over the albumin zone after having sharpened it, had migrated 5.2 cm past the origin. This usually took 4 hours given that there was sufficient volume of electrolyte in both anode and cathode trays to allow maximum passage of current through the gels (50 ma per gel initially falling to 25 ma after 4 hours). It was found that subdivision of transferrin AD into AD_1 and AD_2 was possible with runs of 5.2 to 7.2 cm; however, subdivision of D into D_1, D_2 and D_1D_2 could only be reliably carried out at 5.2 cm or less. At distances of over 5.2 cm only two categories were distinguished, D_1 and D_2, the heterozygotes usually being classified as D_2. After a considerable amount of effort spent in the manipulation of starch concentrations and running distances the subdivision of transferrin D into D_1, D_2 and D_1D_2 was found to be repeatable.

e. Blood typing reagents

1. Anti-J

This was kindly supplied by Dr. K. Bell of the University of Queensland. It gave a titre of $\frac{1}{256}$ with strongly reacting J red cells and after absorption with sheep O cells to remove heterohaemolysin gave a titre of $\frac{1}{1028}$ with sheep R red cells. The highest dilution giving complete haemolysis was $\frac{1}{16}$ for J red cells and $\frac{1}{128}$ for R red cells.

2. Anti-O

Professor C. Stormont generously supplied normal cattle serum containing natural bovine anti-sheep O. This had a titre of $\frac{1}{32}$ against sheep O red cells and gave complete haemolysis at $\frac{1}{8}$. Due to the inconvenience of importing cattle serum from the U.S.A., the sera of 43 goats were tested for the presence of anti-sheep O since Suzuki and Stormont (1961) found the antibody in several goats. Of the goats examined, 3 had anti-R, 6 had anti-O and 34 had neither antibody. Only two animals with anti-O had a titre higher than $\frac{1}{4}$, viz $\frac{1}{16}$ and $\frac{1}{128}$. These were supplied by Dr. G. Alexander from goats used in studies on antibody production in goats carrying sheep-goat hybrid foetuses (Alexander, Williams and Bailey, 1967). Due to the different results obtained using the cattle and goat reagents (Appendix I, p. 132) the goat reagent was not used routinely for detecting Oc substance.

3. Standard red cells

Cow NF68 was selected as a source of strongly reacting J red cells since its red cells gave the same reaction as standard strongly reacting J red cells supplied by Dr. Bell. Two Merino ewes, NF23 and AM1 were used as a source of O and R red cells respectively.

4. Complement

Initially, rabbit serum diluted $\frac{1}{2}$ was used as a source of complement for the anti-J/J haemolytic tests. However, guinea pig serum diluted $\frac{1}{10}$ was found to be equally effective, an observation made previously by Sprague (1958a). For all tests involving sheep red cells guinea pig complement was used, however, the second batch of bovine anti-sheep O received from Professor Stormont was used with rabbit complement at his suggestion since the haemolytic reaction proceeded more rapidly under those conditions than with guinea pig complement. Rabbit serum

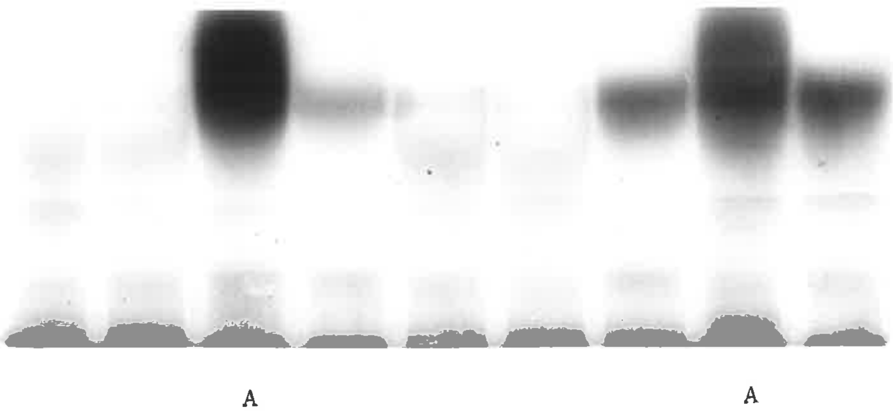
FIGURE 6.

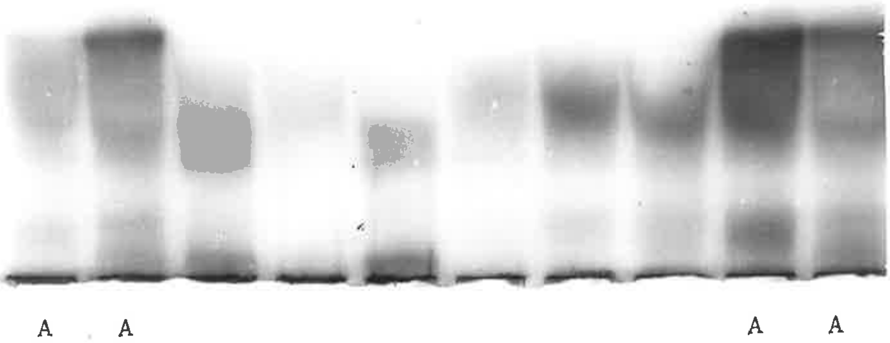
Results of phosphatase staining after electrophoresis of adult cattle sera in:

TOP Ferguson and Wallace's (1961) buffer system.

BOTTOM Gahne's (1963a) buffer system.

Samples that are phosphatase type A are marked, all others are type O. It should be noted that although the two gels do not represent electrophoresis of the same sera, the staining effects are quite distinctive.


A
A


A
A
A
A

used for complement in tests involving sheep red cells was absorbed with sheep red cells to remove heterohaemolysin.

f. The haemolytic test

The haemolytic test for the presence of blood group substances on red cells and in serum has been described in detail by Stone and Irwin (1954) and will not be given in detail here. The actual procedure followed was that described by Sprague (1958a) except that reactions were carried out in perspex plates with 80 chambers 1.6 cm in diameter and 1.0 cm deep as described by Braend (1961). For qualitative inhibition tests where species' antibodies interferred with the tests, 2 ml cattle sera were heated at 100°C for 15 minutes. The coagulated protein was broken up and after centrifuging the supernatant fluid was used as inhibitant. Heating had no detectable effect on qualitative tests for J and Oc substances.

g. Alkaline phosphatase typing

0.08 ml serum was placed into slots prepared by using two laminated bakerlite slot-formers and sera were classified as A or O according to whether they showed A zone activity or not (Figure 6). This classification was not that described by Gahne (1963b) since the buffer system of Ferguson and Wallace (1961) gave markedly different phosphatase staining patterns (Figure 6).

TABLE 5

PHOSPHATASE TYPING OF 18 BULLS AFTER ELECTROPHORESIS IN THE BUFFER SYSTEMS OF GAHNE (1963a) AND FERGUSON AND WALLACE (1961)

	Phosphatase typing	
	A	O
Ferguson and Wallace	2	16
Gahne	6*	12

* Two of these are the same samples that showed A zone activity with Ferguson and Wallace's system.

Table 5 shows the phosphatase typings of 18 bulls using both buffer systems. From the table it would appear that Gahne's system was more suitable for the electrophoresis of serum to be typed for alkaline phosphatase since additional sera were detected with A zone activity. However, it was observed that staining was actually far more intense in Ferguson and Wallace's (1961) system and it was considered that those sera which showed phosphatase activity after electrophoresis in Gahne's system but not in Ferguson and Wallace's actually had a different phosphatase enzyme and not simply a lower concentration of the same A zone. For this reason it seemed profitable to re-examine the association between phosphatase type and J blood group described previously by Rendel and Gahne (1963) and Rendel and Suzuki (1964).

h. Nomenclature for transferrin, J, and phosphatase

The symbolism adopted for cattle transferrins was that proposed by the F.A.O. committee of Ashton _et al._ (1966). Thus, phenotypes are written in the form TfAB, TfA, and TfB and the corresponding genotypes in the form $\underline{Tf}^A/\underline{Tf}^B$, $\underline{Tf}^A/\underline{Tf}^A$ and $\underline{Tf}^B/\underline{Tf}^B$.

A set of zones determined by a single gene is referred to as zone 1, zone 2, zone 3 and zone 4 etc. in decreasing order of mobility.

The nomenclature for the J blood group system is as used by Stone (1962) and Sprague (1958a).

Since alkaline phosphatase variation may not be under simple genetic control only phenotypes are referred to, viz. A and O.

The use of the terms codominant and non-codominant to describe systems showing no dominance (e.g. transferrin) and dominance (e.g. J) is not adopted since the phenomena are adequately described by the latter terms. Codominance is a term which has come into practice with the study of blood-group systems showing no dominance and is actually a misnomer. Thus, whereas it is common practice for blood group alleles $\underline{I}^A$ and $\underline{I}^B$ in man to be regarded as codominant because they show no dominance with respect to each other, they are actually co-dominant with respect to _i_.

III. RESULTS

a. Transferrin

1. Family data

(i) Studies on dam-foetus pairs

Collections of dam-foetus pairs were made at the Metropolitan and Export Abattoirs of South Australia during 15 visits over a period of six months. The number collected at each sampling and information on sex ratio, twins and dead foetuses are given in Table 6. The dead foetuses were usually partly decomposed so that blood sampling was not possible.

Table 7 gives the family data derived from the dam-foetus pairs. The invalid combinations, assuming multiple allelism, are marked with a cross.

In Table 8 the incomplete family data given in Table 7 have been extracted and analysed by the "method of incomplete family data" (Cooper, 1966). This analysis was developed for half sib families where the genotype of the dam was not known. Cooper (1966) showed that regardless of the genotypic frequency in the dams, the offspring of a heterozygous sire ($\underline{Tf}^{i}/\underline{Tf}^{j}$) should show a ratio of 1:1 heterozygotes: homozygotes ($\underline{Tf}^{i}/\underline{Tf}^{j}$: $\underline{Tf}^{i}/\underline{Tf}^{i}$ + $\underline{Tf}^{j}/\underline{Tf}^{j}$) if segregation was normal. Similarly he stated that the ratio of offspring of the type $\underline{Tf}^{i}/\underline{Tf}^{k}$ to those of the type $\underline{Tf}^{j}/\underline{Tf}^{k}$ should also be 1:1 ($\underline{Tf}^{k}$ is any other allele). Although this is correct if $\underline{Tf}^{k}$ is strictly any allele other than $\underline{Tf}^{i}$ or $\underline{Tf}^{j}$; when one includes homozygotes in the offspring such that

TABLE 6

SAMPLES OF COWS AND FOETUSES SHOWING SEX OF FOETUS AND THE OCCURRENCE OF TWINS AND DEAD FOETUSES

Sample	Number of Cows Sampled	Length of Foetuses (Inches)				Total Number of Foetuses		Twins	Dead Foetuses
		≤19"		≥20"					
		M	F	M	F	M	F		
1	10	-		-		10		0	0
2	25	4	8	11	2	15	10	0	0
3	20	7	3	7	2	14	5*	0	1
4	13	4	2	5	2	9	4	1 (M/F)	1
5	19	6	5	4	5	10	10	1 (M/F)	0
6	29	16	7	5	1	21	8*	0	0
7	22	6	9	5	3	11	12	1 (M/F)	0
8	12	3	3	4	3	7	6	1 (M/F)	0
9	23	4	6	9	4	13	10	0	0
10	30	10	10	5	6	15	16	1 (M/M)	0
11	25	5	7	7	7	12	14	1 (M/F)	0
12	25	6	9	4	6	10	15	0	0
13	24	6	7	6	4	12	11	0	1
14	29	9	9	5	6	14	15	0	0
15	30	5	9	7	8	12	17	0	1
Total	336	91	94	84	59	173	153	6	4

* Sex ratio significantly different from 0.5 at 5% level of probability.

TABLE 7

TRANSFERRIN FAMILY DATA AND PHENOTYPIC FREQUENCIES FOR TWO GENERATIONS OF CATTLE (ABATTOIRS MATERIAL)

Dam	Transferrin Type of Foetus										Dams	
	A	AD1	AD2	D1	D2	D1D2	D1E	D2E	AE	E	Total	Frequency
A	41	18	28	X	X	X	X	X	0	X	87	0.27
AD1	16	18	15	4	X	6	1	X	0	X	60	0.18
AD$_2$	20	6	38	X	21	8	X	4	2	X	99	0.29
D_1	X	5	X	4	X	3	0	X	X	X	12	0.04
D_2	X	X	11	X	8	5	X	0	X	X	24	0.07
D_1D_2	X	12	6	1	6	7	1	1	X	X	34	0.10
D_1E	X	2	X	0	X	1	1	0	0	0	4	0.01
D_2E	X	X	1	X	1	1	1	3	1	0	8	0.02
AE	3	1	1	X	X	X	1	0	2	1	9	0.03
E	X	X	X	X	X	X	0	0	0	0	0	0.00
Foetuses Total	80	62	100	9	36	31	5	8	5	1	337	1.00
Foetuses Frequency	0.24	0.18	0.30	0.03	0.11	0.09	0.01	0.02	0.01	0.003	1.00	

Crosses indicate dam-foetal combinations that are not possible assuming the multiple allele hypothesis for the inheritance of transferrin variants.

check this is nonsense writing

$\underline{Tf}^i/\underline{Tf}^k$ can be $\underline{Tf}^i/\underline{Tf}^i$ and $\underline{Tf}^j/\underline{Tf}^k$ can be $\underline{Tf}^j/\underline{Tf}^j$ then the expected genotypic ratio of $\underline{Tf}^i/\underline{Tf}^k$: $\underline{Tf}^j/\underline{Tf}^k$ among the offspring of $\underline{Tf}^i/\underline{Tf}^j$ sires is dependent upon the frequency of $\underline{Tf}^i$ and $\underline{Tf}^j$ in the population and is (1-q)(1-p) where q is the frequency of $\underline{Tf}^j$ and p is the frequency of $\underline{Tf}^i$. If one considers all heterozygous dams of the same transferrin type as a single "sire", the offspring of these dams will have the same relationship with respect to the transferrin locus as half sibs. In Table 8 there is no evidence for a significant departure from expectation in favour of eithe heterozygotes or homozygotes and among the offspring of $\underline{Tf}^i/\underline{Tf}^j$ dams there is no evidence for a significant departure from the expected ratio of 0.5 for $\underline{Tf}^i/\underline{Tf}^k$: $\underline{Tf}^j/\underline{Tf}^k$. These results are discussed further on page 60.

The abattoirs material also provided information on possible maternal-foetal incompatibility involving transferrin type since any heterozygous foetus from a homozygous dam must have one foreign allele. Such foetuses are classed as incompatible. Although the data cannot be tested in the manner of Ashton (1965b) due to the absence of data on the sire's transferrin type, it can be analysed as shown in Table 9. The proportion of offspring in the compatible and incompatible classes is compared with expectation based on the estimated gene frequencies of the four alleles involved - A, D_1, D_2 and E (see Table 15, p. 47). Since the incompatibility mechanism (if any) could operate at a particular stage of development the foetal population was considered in two groups, those 19 inches or less and those 20 inches or greater in length. This subdivision of the foetal population was based on the observation that

TABLE 8

ANALYSES OF INCOMPLETE FAMILY DATA FOR EXPECTED SEGREGATION RATIOS FOR TRANSFERRIN ALLELES USING THE METHOD OF COOPER (1966)

Dam's Genotype Tf^i/Tf^j	Number of Offspring			χ^2_1 (1:1)	P	Number of Offspring			χ^2_1 (1:1)	P
	Heterozygotes	Homozygotes	Total			Tf^i/Tf^k	Tf^j/Tf^k	Total		
Tf^A/Tf^{D1}	18	20	38	0.10	.7-.8	15	7	22	2.9	.05-.1
Tf^A/Tf^{D2}	38	41	79	0.12	.7-.8	8	12	20	0.8	.3 -.5
Tf^{D1}/Tf^{D2}	7	7	14	0.00	1.0	13	7	20	1.8	.1 -.2
Tf^{D1}/Tf^E	1	0	1	-	-	3	0	3	-	0.5*
Tf^{D2}/Tf^E	3	1	4	-	0.63*	2	2	4	-	1.0*
Tf^A/Tf^E	2	4	6	-	0.69*	2	1	3	-	1.0*
Total	69	73	142	0.11	.7-.8	43	29	72	2.72	.05-.1

* Exact probabilities calculated using binomial.

where all the dams of the same breed

TABLE 9

MATERNAL-FOETAL INCOMPATIBILITY : SIGNIFICANCE TESTS ON ABATTOIRS DATA

[illegible]

Homozygous Dam	All Offspring					Offspring $\leqslant$19 inches					Offspring $>$20 inches				
	Compatible		Incompatible		Total	Compatible		Incompatible		Total	Compatible		Incompatible		Total
	O	E$	O	E$		O	E$	O	E$		O	E$	O	E$	
TfA	41	43.50	46	43.50	87	24	25.00	26	25.00	50	17	18.50	20	18.50	37
TfD_1	4	2.11	8	9.89	12	2	1.23	5	5.77	7	2+	0.88	3*	4.12	5
TfD_2	8	7.05	16	16.95	24	4	3.82	9	9.18	13	4+	3.23	7*	7.77	11
Total	53	52.66	70	70.34	123	30	30.05	40	39.95	70	23	22.61	30	30.39	53
χ^2_1	0.004, $.95 < P < .98$					0.0002, $.98 < P < .99$					0.001, $.90 < P < .95$				
Homogeneity Test	$\chi^2_2 = 1.97$, $.3 < P < .5$					$\chi^2_2 = 1.90$, $.3 < P < .5$					$\chi^2_1 = 0.07$, $.7 < P < .8$				

* and + These classes pooled for the homogeneity test.

$ Expectations are based on the estimates of gene frequency in Table 15.

dead foetuses found were usually about 12 inches long or less and that subdivision at the 20 inch level gave approximately equal numbers of foetuses in the two classes. There was no heterogeneity within compatibility class between transferrin types of the dams so the χ^2 test on observed and expected numbers in compatibility classes was carried out on the totals of these classes. There was no significant overall deficiency of foetuses in the incompatible category. This observation is discussed in relation to the published results of other workers on page 62.

In Table 10 the transferrin types of dams with their twins are presented. The absence of a dam of type A in a sample of 6 carrying twins is not statistically significant (P = 0.15) and the observation of 5 unlike-sex pairs of twins in a sample of 6 is also a non-significant departure from expectation (P = 0.22). All members of the twin pairs have the same transferrin type which is not surprising in view of the observation that all twins showed venous anastomosis *in utero*. The probability that all pairs are the same due to chance is 0.0044. Four dams carried dead foetuses which were decomposing and could not be bled. Three of the dams were transferrin type A and one was type AD_2, the probability of obtaining such a distribution in a sample of four being 0.062.

[illegible] pressing

TABLE 10

THE TRANSFERRIN AND J-Oc TYPES OF DAMS AND THEIR TWIN FOETUSES

DAM		FOETUSES			
JOc	Tf	Sex	Length	Tf*	JOc*
J	AD_1	M/F	27"	AD_2	Oc
Oc	D_1D_2	M/F	17"	D_1D_2	Oc
Oc	AD_2	M/F	12"	AD_2	Oc
J	AD_2	M/F	39",36"	D_2	JOc
J	AD_2	M/M	9"	D_2E	JOc
J	D_1D_2	M/F	23"	AD_2	JOc

* all members of twin pairs had the same Tf and JOc phenotype

(ii) Half-sib families

All members of 51 half-sib families were blood-sampled for studies on the association of transferrin type with production parameters (see Section B, p. 95). The sires and dams of these animals were not bled because a large proportion were dead or had been sold at the time of sampling. The transferrin types of the offspring in each progeny group (half-sib family) of Friesian and Jersey cows are given in Table 11 with the most likely phenotype of the sire. Although the inference of homozygous sires can only be made at a certain level of probability, the genotype of heterozygous sires can be inferred exactly since the presence of both homozygotes in the progeny is conclusive evidence for heterozygosity. Thus half-sib families from heterozygous sires in Table 11 can be analysed for the expected segregation of transferrin alleles using "the method of incomplete family data" (Cooper, 1966) and this is shown in Table 12. It is assumed that there are no errors in the parentage records. The 34 progeny groups are not heterogeneous with respect to the proportion of heterozygotes and homozygotes present and the probability of obtaining three significant χ^2_1 values in a series of 34 is 0.25. Thus the occurrence of three significant departures from a 1:1 ratio might be expected by chance. When the totals are examined it can be seen that there are actually more homozygotes than heterozygotes although the difference is not significant. An excess of homozygous offspring might be expected if some of the presumed heterozygous sires were in fact homozygous, the inference being incorrect due to incorrect parentage records. An attempt was made to determine the extent to which incorrect parentage records could have contributed to such misclassification. This was done by comparing the frequency of presumed sires' phenotypes among the 51 sires with the frequency of phenotypes in the sample of cows (Table 13). In both the Jersey and Friesian data the phenotypic frequencies among the sires differ from those in cows, heterozygotes being in higher frequency in the bulls than in cows. In the case of the Jersey breed the differences in phenotypic frequencies

[illegible] [illegible] nbrs, small

TABLE 11

THE TRANSFERRIN TYPES OF 36 JERSEY AND 15 FRIESIAN HALF-SIB FAMILIEIS, TOGETHER WITH THE INFERRED TRANSFERRIN TYPES OF THE SIRES

Progeny Group	Transferrin Type					
	A	AD	D	DE	AE	Sire
1	2	7	4	0	0	AD
2	16	12	4	0	0	AD
3	5	11	5	0	0	AD
5	10	7	8	0	0	AD
7	1	3	6	0	0	AD
8	1	8	2	0	0	AD
9	2	5	4	0	0	AD
11	3	12	2	0	0	AD
13	3	7	4	0	0	AD
14	3	7	3	0	0	AD
15	4	4	1	0	0	AD
16	5	3	2	0	0	AD
18	6	8	2	0	1	AD
21	5	8	5	0	0	AD
22	4	5	4	0	0	AD
24	14	15	4	0	0	AD
25	5	6	1	0	0	AD
26	3	4	3	0	0	AD
28	7	10	1	0	0	AD
30	4	6	1	0	0	AD
31	13	9	9	0	0	AD
32	5	6	1	0	0	AD
33	3	10	4	0	0	AD
34	2	6	5	0	0	AD
4	8	1	0	0	0	A
6	3	10	0	0	0	A
10	1	8	0	0	0	A
12	5	7	0	0	0	A
17	3	7	0	0	0	A
19	5	7	0	0	1	A
20	5	7	0	0	0	A
23	8	4	0	0	0	A
27	3	8	0	0	0	A
29	3	8	0	0	0	A
35	16	17	0	0	0	A
36	11	2	0	0	0	A
Total for Phenotypes	197	265	85	0	2	
Total	549					

Progeny Group	Transferrin Type					
	A	AD	D	DE	AE	Sire
1	2	6	1	0	0	AD
2	3	6	1	0	0	AD
3	4	10	6	0	0	AD
4	1	4	6	1	0	AD
5	8	9	4	0	0	AD
6	2	16	5	0	0	AD
8	3	6	3	1	0	AD
9	1	24	9	1	0	AD
10	3	8	2	0	3	AD
13	12	15	2	0	0	AD
11	11	3	0	0	0	A
15	8	6	0	0	0	A
12	13	9	0	0	0	A
14	0	10	12	0	0	D
7	0	2	6	3	1	DE
Total for Phenotypes	71	134	57	6	4	
Total	272					

TABLE 12

NUMBERS OF HOMOZYGOTES AND HETEROZYGOTES FOR $\underline{Tf}^{A}$ AND $\underline{Tf}^{D}$ IN THE PROGENY OF HETEROZYGOUS SIRES

Sire	Number of Offspring			χ^2_1	P	Homogeneity χ^2_{33}
	Heterozygous[†]	Homozygous[†]	Total			
J1	7	6	13	0.08		36.83 (.2 < P < .3)
J2	12	20	32	2.00		
J3	11	10	21	0.05		
J5	7	18	25	4.84	.02-.05	
J7	3	7	10	1.60		
J8	8	3	11	2.27		
J9	5	6	11	0.09		
J11	12	5	17	2.88		
J13	7	7	14	0.00		
J14	7	6	13	0.08		
J15	4	5	9	0.11		
J16	3	7	10	1.60		
J18	8	8	16	0.00		
J21	8	10	18	0.22		
J22	5	8	13	0.69		
J24	15	18	33	0.27		
J25	6	6	12	0.00		
J26	4	6	10	0.40		
J28	10	8	18	0.22		
J30	6	5	11	0.09		
J31	9	22	31	5.45	.01-.02	
J32	6	6	12	0.00		
J33	10	7	17	0.53		
J34	6	7	13	0.08		
F1	6	3	9	1.00		
F2	6	4	10	0.40		
F3	10	10	20	0.00		
F4	4	7	11	0.82		
F5	9	12	21	0.43		
F6	16	7	23	3.52		
F8	6	6	12	0.00		
F9	24	10	34	5.77	.01-.02	
F10	8	5	13	0.69		
F13	15	14	29	0.03		
Total	283	289	572	0.21	.5 -.7	

[†] Heterozygous and homozygous with respect to $\underline{Tf}^{A}$ and $\underline{Tf}^{D}$ only. $\underline{Tf}^{D}$ has not been subdivided into $\underline{Tf}^{D1}$ and $\underline{Tf}^{D2}$.

TABLE 13

THE FREQUENCY OF INFERRED TRANSFERRIN TYPES IN JERSEY AND FRIESIAN BULLS COMPARED WITH THE OBSERVED FREQUENCIES IN COWS

Animals	Total	Transferrin Type (Bulls Inferred)									
		Number					Frequency				
		A	AD	D	AE	DE	A	AD	D	AE	DE
Jersey Bulls +	36	12	24*	0	0	0	0.33	0.67	0.00	0.00	0.00
Jersey Cows +	548	197	264	85	2	0	0.36	0.48	0.16	0.004	0.00
Friesian Bulls	15	3	10*	1	0	1	0.20	0.67	0.07	0.00	0.07
Friesian Cows	272	71	134	57	4	6	0.26	0.49	0.21	0.02	0.02

* The transferrin type of heterozygous bulls is inferred exactly (Cooper, 1966).

\+ The genotypic frequencies in these two populations are significantly different

$\chi^2_2 = 8.03, \quad 0.01 < P < 0.02$

are significant. This is discussed on page 63.

2. Population data

(i) Abattoirs material

The results of studies on abattoirs material presented in the form of incomplete family data in Table 7 were also used for population studies based on the assumption that the abattoirs sample represented a random sample from a large interbreeding population of cattle. Thus the genotypic frequencies among the dams should be the same as those among their foetuses assuming random mating and Hardy-Weinberg equilibrium. Similarly, the genotypic frequencies among old ($\geqslant 20''$) and young ($\leqslant 19''$) foetuses should be the same as each other and the same as their dams if there is no differential viability of transferrin genotypes. Table 14 shows the results of homogeneity tests comparing genotypic frequencies in these subpopulations. None is significant. Similarly, gene frequencies are compared in Table 15.

The observed transferrin genotypic frequencies and those expected assuming random mating and Hardy-Weinberg equilibrium are presented in Table 16, old and young foetuses being considered together and separately. No significant departure from random mating expectation was observed. The findings summarized in Table 14, 15 and 16 are discussed on page 64.

TABLE 14

HOMOGENEITY TESTS COMPARING TRANSFERRIN GENOTYPIC FREQUENCIES* BETWEEN SUB-POPULATIONS OF DAMS AND THEIR FOETUSES

Sub-Populations Compared	χ^2_8 Homogeneity	P	Numbers in Compared Sub-Populations	
Dams and foetuses	6.4	.5 -.7	335	337
Dams and foetuses ≤19" long	4.8	.7 -.8	186	185
Dams and foetuses ≥20" long	2.4	.95-.98	149	152
Foetuses ≤19" long and foetuses ≥20" long	9.4	.3 -.5	186	149

* Only 9 genotypes were considered, $\underline{Tf}^E/\underline{Tf}^E$ individuals being pooled with the $\underline{Tf}^A/\underline{Tf}^E$ class for this analysis.

TABLE 15

TRANSFERRIN GENE FREQUENCIES FOR TWO GENERATIONS OF CATTLE AND THEIR HOMOGENEITY*

Transferrin Gene		Total Sample		Subdivision of Sample						Estimate of Gene Frequency Based on Dams + Foetuses
				Foetuses ⩽19"		Foetuses ⩾20"		Foetuses Only		
		Dams	Foetuses	Dams	Foetuses	Dams	Foetuses	⩽19"	⩾20"	
A		0.52	0.49	0.53	0.51	0.50	0.46	0.51	0.46	0.5000
D_1		0.18	0.17	0.16	0.16	0.19	0.19	0.16	0.19	0.1756
D_2		0.28	0.31	0.27	0.30	0.29	0.32	0.30	0.32	0.2939
E		0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.0301
Number Examined		335	337	186	185	149	152	186	149	672
Homogeneity Test*	χ^2_3	2.6		0.44		0.64		0.85		
	P	0.3 - 0.5		0.9 - 0.95		0.8 - 0.9		0.8 - 0.9		

*Homogeneity tests were based on the actual number of genes in the compared sub-populations.

TABLE 16

COMPARISON OF OBSERVED TRANSFERRIN GENOTYPIC FREQUENCIES AND THOSE EXPECTED UNDER CONDITIONS OF RANDOM MATING FOR ABATTOIRS MATERIAL

SUB-POPULATIONS	NUMBER OF INDIVIDUALS	χ^{2*}_{6}	P
Dams	335	1.72	.90-.95
Foetuses	337	5.84	.3 -.5
Dams	186	0.84	>0.99
Foetuses <19"	185	4.91	.5 -.7
Dams	149	0.80	>0.99
Foetuses >20"	152	5.86	.3 -.5

* degrees freedom = number classes (10) - number independent parameters estimated from data (3)-1

(ii) Dairy farm sample

The gene frequency estimates based on Table 11 (p. 45) are shown in Table 17. The D phenotype is now known to be separable into D_1 and D_2 so the frequency given for $\underline{Tf}^D$ represents the total frequency of $\underline{Tf}^{D1} + \underline{Tf}^{D2}$. Thus the frequency given for $\underline{Tf}^D$ in Table 17 should be considered with reference to Table 20 (p. 48). The difference between Jersey and Friesian cattle in this study and in others reported is the extremely low frequency of the $\underline{Tf}^E$ allele in Jersey cattle.

From the gene frequencies in Table 17 the expected frequency of the five observed genotypes has been determined and are presented in Table 18. There is almost exact agreement between the observed frequency of the genotypes and that expected under conditions of random mating.

Table 19 gives the transferrin typings of one Jersey and one Friesian stud herd and eighteen bulls of both breeds from the Artificial Breeding Centre. From these the gene frequencies in Table 20 were derived and are there compared with the published data.

3. A new type of transferrin variant in cattle

During the routine typing of sera from bulls at the Artificial Breeding Centre it was noted that a bull typed as TfDE in tris-citrate buffer had a transferrin pattern in which the slowest E zone was slightly faster than that of other animals. Autoradiography revealed that this zone specifically bound ferric iron (Figure 7b) and electrophoresis in tris-cacodylate buffer indicated that the transferrin pattern why just these references

TABLE 17

TRANSFERRIN GENE FREQUENCIES FOR JERSEY AND FRIESIAN CATTLE IN SOUTH AUSTRALIA TOGETHER WITH OTHER REPORTED FREQUENCIES

Breed	Source	Number Animals	Transferrin Gene		
			A	D	E
Jersey	South Australia	549	0.60	0.40	0.002
	Ashton & Fallon (1962) (Australia)	375	0.54	0.46	0.00
	Osterhoff (1964) (S. Africa)	276	0.68	0.32	0.00
Friesian	South Australia	272	0.51	0.47	0.02
	Ashton & Fallon (1962) (U.K.)	179	0.52	0.45	0.03
	Datta & Stone (1963) (U.S.A.)	-	0.46	0.53	0.01

TABLE 18

THE OBSERVED AND EXPECTED FREQUENCIES OF TRANSFERRIN GENOTYPES ASSUMING RANDOM MATING WITH RESPECT TO THE TRANSFERRIN LOCUS

Breed	Details	Transferrin Genotype				
		A	AD	D	AE	DE
Jersey	Observed number	197	265	85	2	0
	Observed frequency	0.36	0.48	0.16	0.004	0
	Expected frequency*	0.36	0.48	0.16	0.002	0.002
Friesian	Observed number	71	134	57	4	6
	Observed frequency	0.26	0.49	0.21	0.015	0.022
	Expected frequency*	0.26	0.48	0.22	0.02	0.02

* Expectations based on random mating using gene frequencies in Table 17.

TABLE 19

TRANSFERRIN PHENOTYPES OF PUREBRED JERSEY AND FRIESIAN CATTLE FROM VARIOUS SOURCES WHERE D HAS BEEN SCORED AS D_1 OR D_2

Breed	Source	Number	Phenotype								
			A	AD_1	AD_2	D_1	D_2	D_1D_2	D_1E	D_2E	AE
Jersey	Roseworthy	50	15	5	20	0	7	3	0	0	0
	A.B. Centre	10	3	1	4	0	2	0	0	0	0
	Total	60	18	6	24	0	9	3	0	0	0
	Frequency		0.30	0.10	0.40	0.00	0.15	0.05	0.00	0.00	0.00
Friesian	Anama	22	2	8	1	5	0	5	1	0	0
	A.B. Centre	8	0	1	2	1	3	1	0	0	0
	Total	30	2	9	3	6	3	6	1	0	0
	Frequency		0.07	0.30	0.10	0.20	0.10	0.20	0.03	0.00	0.00

TABLE 20

TRANSFERRIN GENE FREQUENCIES FOR PUREBRED JERSEY AND FRIESIAN CATTLE FROM VARIOUS SOURCES WHERE D HAS BEEN SCORED AS D_1 OR D_2

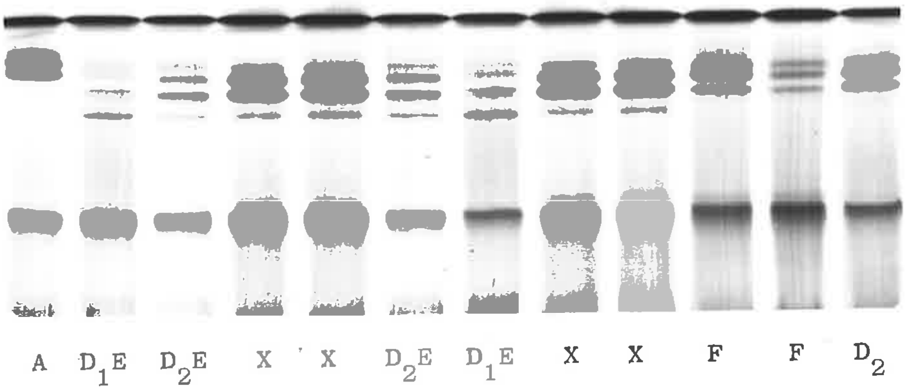
Breed	Source	Number Animals	Transferrin Alleles			
			A	D_1	D_2	E
Jersey	This Thesis (Table 19)	60	0.55	0.08	0.38	0.00
	Ashton (1965) (Australia)	578	0.72	0.05	0.23	0.00
	Jamieson (1966) (U.K.)	54	0.69	0.12	0.20	0.00
Friesian	This Thesis (Table 19)	30	0.27	0.47	0.25	0.02
	Ashton (1965) (Australia)	30	0.48	0.10	0.37	0.05
	Kristjansson (1965 Canada)	-	0.62	0.13	0.16	0.09
	Jamieson (1966) (U.K.)	179	0.47	0.22	0.26	0.05

FIGURE 7.

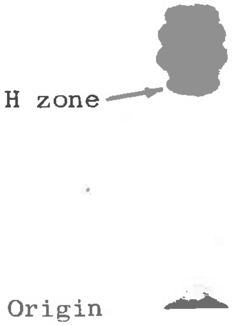
a. The transferrin pattern of Bull "X" compared with a number of standard transferrin types. Note that the H zone of Bull "X" is slightly faster than the slowest zone of D_1E and D_2E .

b. Autoradiograph showing that the H zone of Bull "X" specifically binds iron. The pattern here is somewhat different from that shown in Figure 7a because electrophoresis of labelled transferrin was carried out in the buffer system of Ferguson and Wallace (1961).

a



b



was not typical for D_2E(Figure 7a). The sera of 21 offspring of this bull and their dams were collected and typed on starch gel and checked by autoradiography for their ability to bind ferric iron. The transferrin typings of the offspring and their dams are shown in Table 21. The slow zone (called H) could not be detected in all offspring and it was found that the transferrins of all offspring could be typed normally without regard to the slow zone. If this H zone was actually the slowest member of a regular zone quartet with its fastest zones coincident with the slowest zones of transferrin A (as is the case with D_2E) one would expect to find individuals of type AH in the offspring, and these were not found. From his offspring, the bull's genotype may be inferred as $\underline{Tf}^{D2}/\underline{Tf}^{D2}$. In Figure 8 is shown the result of electrophoresis of the sera of a number of offspring and dams. Three of the offspring have an obvious H zone. The transferrin pattern of calf number one (Figure 17, p. 56) also shows a slow zone in the H region. The implications of these observations is discussed later (p. 63).

b. Blood group substances

1. Population data for the J blood group system

(i) Dairy farm sample

The 51 half sib families used in Section B of this thesis for studies on the association between the J blood group and production characters in dairy cattle are tabulated in Table 22. For each progeny group in both breeds the number of animals of phenotype j^a, composed of those with and without natural anti-J in their sera, and the

Why not D_2 E

TABLE 21
FAMILY DATA ON THE INHERITANCE OF A SLOW MINOR TRANSFERRIN BAND IN JERSEY CATTLE

SIRE "X" TRANSFERRIN TYPE D_2D_2H

Sire D_2 E

DAM	OFFSPRING	PRESENCE OF WEAK H BAND	
D_1D_2	D_1D_2	+	D_1E_1
AD_1	AD_2	+	AE_1 indistinguishable from AD_2+X
A	AD_2	+	AE_1
A	AD_2	+	AE_1 ''
AD_2	AD_2	+	AE_1
AD_1	D_1D_2	+	D_1E_1 14 – 8
D_1D_2	D_1D_2		D_1D_2
AD_2	AD_2		AD_2
Dead	AD_2		AD_2 ?
D_1D_2	D_2	+	D_2E_1 } indistinguishable from D_2+X
D_1D_2	D_2	+	D_2E_1
N.T.	D_2	+	D_2E_1
AD_2	AD_2	+	A_1E_1 indist from AD_2+X
D_1D_2	D_1D_2		D_1D_2
AD_1	D_1D_2		D_1D_2
AD_2	D_2	+	D_2E_1
D_2	D_2	+	D_2E_1
AD_2	D_2	+	D_2E_1
N.T.	AD_2	+	AE_1
N.T.	D_1D_2		D_1D_2
N.T.	D_2E		D_2E
N.T.	D_2		D_2

N.T. = Not tested

FIGURE 8.

Electrophoresis of the serum from four offspring from Bull "X" and five of the cows to which the bull was mated.

Samples 4 and 9 are AD_1 and AD_2 standards respectively and sample 12 shows the transferrin pattern of Bull "X".

Samples 1, 2, 3 and 5 are some of the progeny of this bull and are transferrin types D_2, D_2, AD_2 and D_1D_2 respectively. Note that the first three have an obvious slow zone in the same position as that of Bull "X". This zone was shown to be transferrin in all those animals that had it. Some others with no obvious zone in this region still showed faint fogging of the X-ray plate and were scored as positive for the H zone.

Samples 6, 7, 8, 10 and 11 are five cows to which the bull was mated.

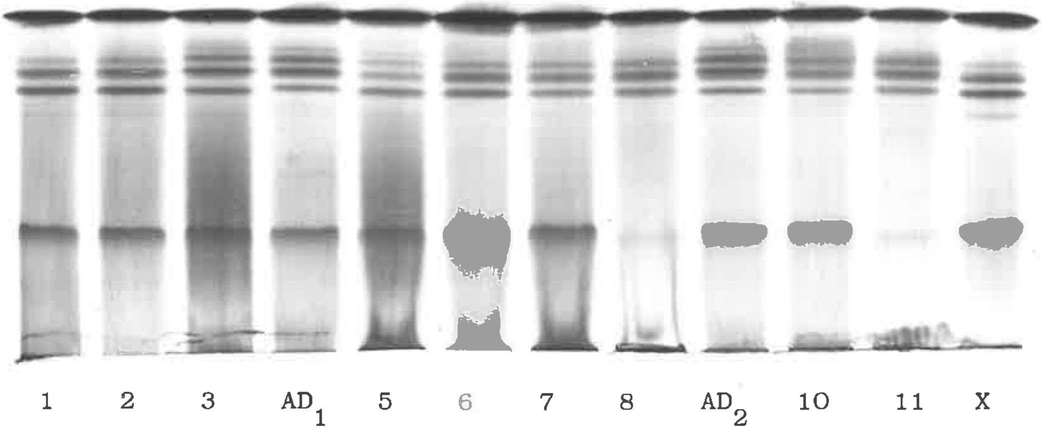

1
2
3
AD_1
5
6
7
8
AD_2
10
11
X

TABLE 22

THE J INHIBITION TYPES OF ALL JERSEY AND FRIESIAN COWS SAMPLED

Progeny Group	J inhibition type			
	J^a			J^S+J^{CS}
	A[≠]	H[≠]	Total	
1	3	5	8	5
2	15	11	26	6
3	8	6	14	7
4	4	3	7	2
5	4	10	14	11
6	3	4	7	6
7	1	2	3	7
8	1	0	1	10
9	3	2	5	6
10	0	1	1	8
11	5	6	11	6
12	1	3	4	8
13	0	8	8	6
14	5	2	7	6
15	1	3	4	5
16	0	2	2	8
17	1	6	7	3
18	7	4	11	6
19	4	3	7	6
20	3	1	4	8
21	5	5	10	8
22	7	3	10	3
23	6	6	12	0
24	4	10	14	19
25	2	0	2	10
26	3	1	4	6
27	0	3	3	8
28	15	2	17	1
29	5	0	5	6
30	3	0	3	8
31	6	13	19	12
32	7	4	11	1
33	1	14	15	2
34	9	3	12	1
35	6	4	10	23
36	2	6	8	5
Total	150	156	306	243
Frequency	0.27	0.28	0.56	0.44

Progeny Group	J inhibition type			
	J^a			J^S+J^{CS}
	A[≠]	H[≠]	Total	
1	2	7	9	0
2	1	7	8	2
3	4	9	13	7
4	5	4	9	3
5	9	4	13	8
6	11	8	19	4
7	1	3	4	8
8	2	0	2	11
9	2	0	2	33
10	4	2	6	10
11	3	7	10	4
12	2	10	12	10
13	7	9	16	13
14	3	1	4	18
15	6	6	12	2
Total	62	77	139	133
Frequency	0.23	0.28	0.51	0.49

[≠] A = sera containing no detectable J substance or anti - J haemolysin

H = sera containing anti - J haemolysin and no detectable J substance number of animals with J substance in their sera are presented. The latter class includes both the J^{cs} and J^{s} phenotypes of Stone (1962) and the J and JOc phenotypes of Sprague (1958a). These were not distinguished. The frequency of the phenotypes is very similar in both breeds, the differences not being significant ($X^2_2 = 2.30$). The hi

(ii) Abattoirs sample

In Table 23 are set out the numbers and frequencies of the four J-Oc inhibition types. Both dams and foetuses were tested for the independent occurrence of J and Oc substances in the populations and the results are compared with those of Sprague (1958a). Because the foetal sera showed a higher than usual proportion of inhibitions that resulted in partial haemolysis of the standard cells, two sets of foetal data are presented. The first includes all positive inhibitions and the second omits titres of less then 1 as positive inhibition. Sprague's (1958a) data showed a significant positive association between the occurrence of the J and Oc substances in two populations of cattle but not in a third. This might be expected if the frequency of the $\underline{J}^{JOc}$ allele was low. In the foetal data examined here there is also a significant positive association between the occurrence of the J and Oc substances regardless of which set of foetal data is considered. There is a very low proportion of foetuses in the inhibition class "J" compared with the dams and other published data. When the data on the dams is considered, there is significant heterogeneity in the occurrence of the two antigens; however, in contrast with the foetal material and

bad lang.

TABLE 23

THE NUMBER AND FREQUENCY OF THE FOUR INHIBITION TYPES IN CATTLE FOETUSES AND A NUMBER OF POPULATIONS OF ADULTS

Population	Details	Inhibition Class				Total	Contingency Test$	
		JOc	Oc	J	–		χ^2_1	Probability
Foetuses	All inhibitions	161	150	3	23	337	15.54	$P < .001$
	Frequency	0.48	0.45	0.009	0.07	1		
Foetuses	Omitting weak reactions ⌿	135	145	11	46	337	16.13	$P < .001$
	Frequency	0.40	0.43	0.03	0.14	1		
Dams	Number	39	83	170	23	315	105.42	$P < .001$
	Frequency	0.12	0.26	0.54	0.07	1		
U.C. Beef	Sprague (1958a)	0.06	0.13	0.21	0.60	453	2.24*	$.1 < P < .2$
Aberdeen Angas		0.39	0.30	0.29	0.01	224	33.00*	$P < .001$
Jersey		0.49	0.40	0.11	0.00	354	29.50*	$P < .001$

⌿ Haemolysis scores of 2 or more with undiluted inhibiting serum are considered weak reactions.

$ Test for the independent occurrence of J and Oc substances in a population of cattle.

* Writer's calculation.

other published data, there is a negative association between the two. That is, there are more individuals in the classes "J" and "Oc" than might be expected assuming independent occurrence of the two characters. This observation is discussed in relation to the genetic control of the J-Oc blood-group system on page 75.

2. Serum alkaline phosphatase and the Oc substance

dams or mated

The abattoirs cattle that were typed for the presence of J and Oc substances in their sera were also typed for the presence or absence of the A phosphatase zone. It can be seen from Table 24 that there is no significant association between the presence in cattle serum of the Oc substance and alkaline phosphatase isozyme A.

TABLE 24

TEST FOR AN ASSOCIATION BETWEEN THE PRESENCE OF THE A PHOSPHATASE ISOZYME AND THE Oc SUBSTANCE IN CATTLE SERUM

A phosphatase does not occur in young calves

Obs

		A PHOSPHATASE		TOTAL
		Present	Absent	
Oc SUBSTANCE	Present	29	90	119
	Absent	45	138	183
TOTAL		74	228	302

$\chi^2_1 = 0.002$ $\quad .98 > P \geqslant .95$

3. Blood group substances in seminal plasma

In man, blood-group substances are present in the seminal plasma of secretors but not in that of non-secretors (Hartmann, 1941). However, the concentration of A, B and H substances in the seminal plasma of secretors is higher than in their saliva (Boettcher, personal communication 1967).

It is apparent from Table 25 that all bulls examined had Oc substance in their seminal plasma irrespective of their blood serum phenotype. This observation is similar to that made on the saliva of sheep by Rendel (1957) except that sheep of group 1 did not have O substance in their saliva whereas cattle with the corresponding phenotype "-" can have Oc substance in their seminal plasma. This is discussed on page 74.

c. J-Oc substances and serum proteins in foetuses and calves

1. Blood group substances and transferrin in foetal serum

(i) Transferrin

When the transferrins of dams and foetuses collected at the abattoirs were first typed in tris-citrate without regard to the subdivision of transferrin D into D_1 and D_2 more exceptions to the allelic hypothesis were noted than one might expect to find even in dairy farm material. It was concluded therefore, that the phenotypes observed in the foetuses did not represent the same genotypes as the corresponding phenotypes in adults. For this reason the transferrin patterns of foetuses of various lengths were examined more thoroughly. In Figure 9 the transferrin patterns of foetuses of transferrin type D_2

TABLE 25

J-Oc INHIBITION TYPES OF BULLS DEPENDING UPON WHETHER BLOOD-SERUM OR SEMINAL PLASMA WAS USED AS INHIBITANT

BULL	BLOOD SERUM	SEMINAL PLASMA
G.P.C.	Oc	Oc
H.I.D.	Oc	Oc
S.S.M.	Oc	Oc
A.M.J.	"-"	Oc
G.S.S.	"-"	Oc
J.M.E.	*J	JOc
M.P.P.	*J	JOc
S.G.S.	*J	JOc
H.C.S.	*J	JOc
Y.E.C.	J	JOc
F.R.2.	J	JOc

* When the cells of these were tested they had J substance on them and so were actually J^{cs}.

FIGURE 9.

a. and b. Serum transferrin patterns of foetuses of various lengths and an adult of transferrin type D_2.

a. In tris-cacodylate

b. In tris-citrate

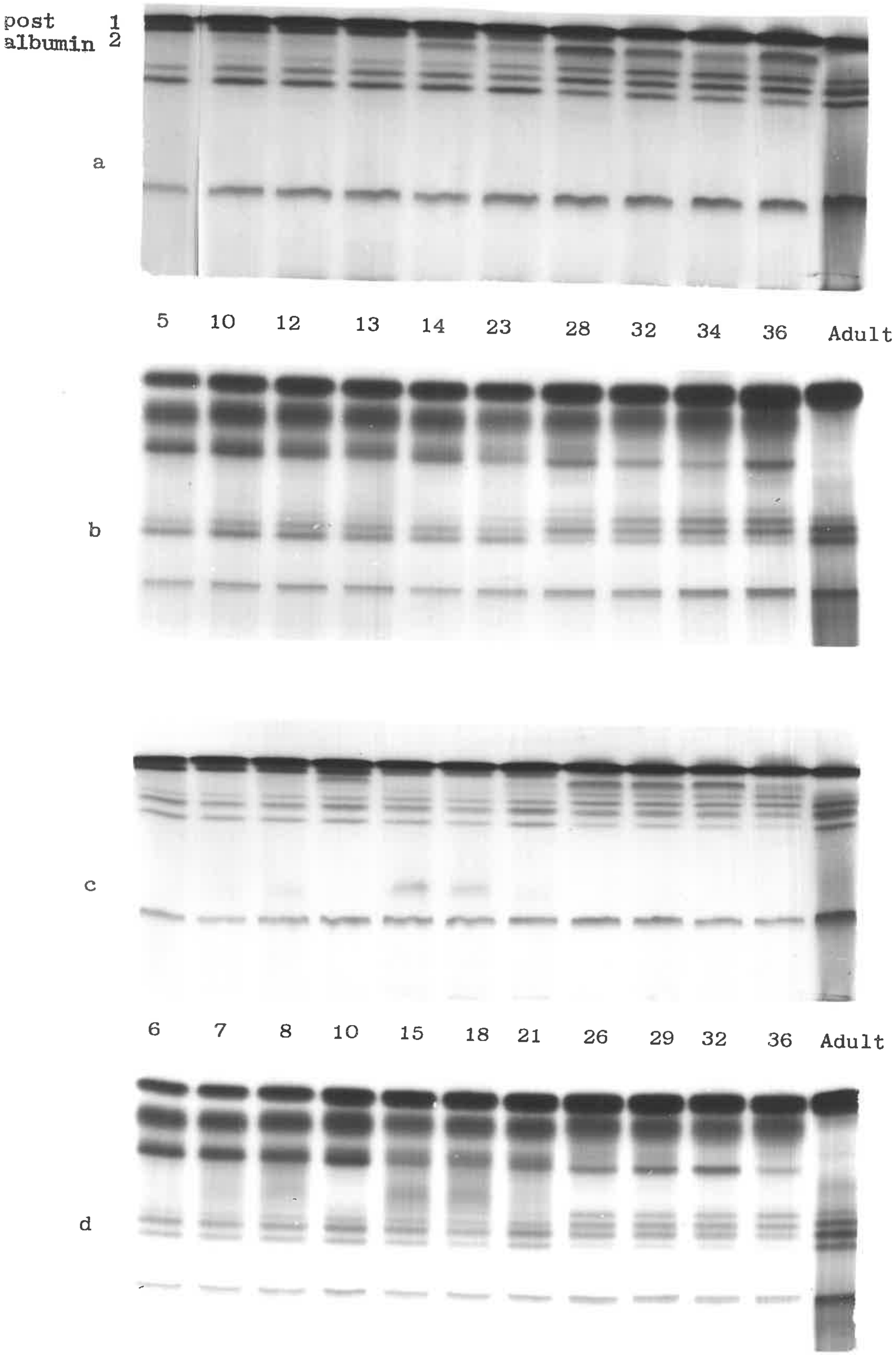
Sample	Length of foetus (ins.)
1	5
2	10
3	12
4	13½
5	14½
6	23
7	28
8	32
9	34
10	36
11	Adult

c. and d. Serum transferrin patterns of foetuses of various lengths and an adult of transferrin type AD_2.

c. In tris-cacodylate

d. In tris-citrate

Sample	Length of foetus (ins.)
1	6
2	7
3	8
4	10
5	15½
6	18
7	21
8	26
9	29
10	32
11	36
12	Adult


post 1
albumin 2
a
5 10 12 13 14 23 28 32 34 36 Adult
b
c
6 7 8 10 15 18 21 26 29 32 36 Adult
d

and AD_2 are shown after electrophoresis in two types of buffer system. From a knowledge of transferrin patterns in adults only, the first six samples in Figure 9(d) might be classified as D_2, not AD_2; however, an examination of the transferrin patterns obtained using tris-cacodylate as the gel buffer (9c) reveals that in these samples the second slowest zone may be resolved into two zones indicating that the transferrins are type AD_2. This criterion was not possible with $TfAD_1$ at normal running temperatures (Figure 10a); however, if one considers the transferrin patterns obtained in tris-cacodylate for TfD_1 (Figure 10b), TfD_2 (Figure 9a) and TfA (Figure 10c) and relates the relative staining intensities of the transferrin zones of the quartet to the presence or absence of post-albumin zones 1 and 2, then for all these types, where the predominant post-albumin zone is zone 1, the slowest transferrin zone is the major one. Thus, foetal transferrins may be readily typed by taking the relative intensities of the foetal post-albumin zones into account. Where post-albumin zone 1 is predominant, homozygotes ($\underline{Tf}^{D1}/\underline{Tf}^{D1}$; $\underline{Tf}^{D2}/\underline{Tf}^{D2}$; $\underline{Tf}^{A}/\underline{Tf}^{A}$) have their slowest transferrin zone as the major zone (also $\underline{Tf}^{D1}/\underline{Tf}^{D2}$; Figure 10d). However, heterozygotes ($\underline{Tf}^{A}/\underline{Tf}^{D1}$; $\underline{Tf}^{A}/\underline{Tf}^{D2}$) have their two slowest zones of about equal intensity since the second slowest zone in heterozygotes is the major zone of the TfA phenotype. No difficulty occurred with the typing of TfD_1E, TfD_2E and TfAE whose patterns are given in Figure 11 (a and b).

A consideration of the transferrin patterns of homozygous foetuses reveals that the trend from a pattern in young foetuses

FIGURE 10.

Serum transferrin patterns of foetuses of various lengths and adults of transferrin types:

a. AD_1

b. D_1

c. A

d. D_1D_2

in tris-cacodylate.

Sample	Length of foetus (ins.)			
	a.	b.	c.	d.
1	6½	8½	5½	6
2	7½	9½	7	7
3	10	12	10	10
4	13	25	12½	14½
5	15½	27½	15	18
6	17	30	18½	20
7	24	34	24	23½
8	28	36	29	28
9	31	Adult	34	32
10	33		38	35
11	36		Adult	40
12	Adult			Adult

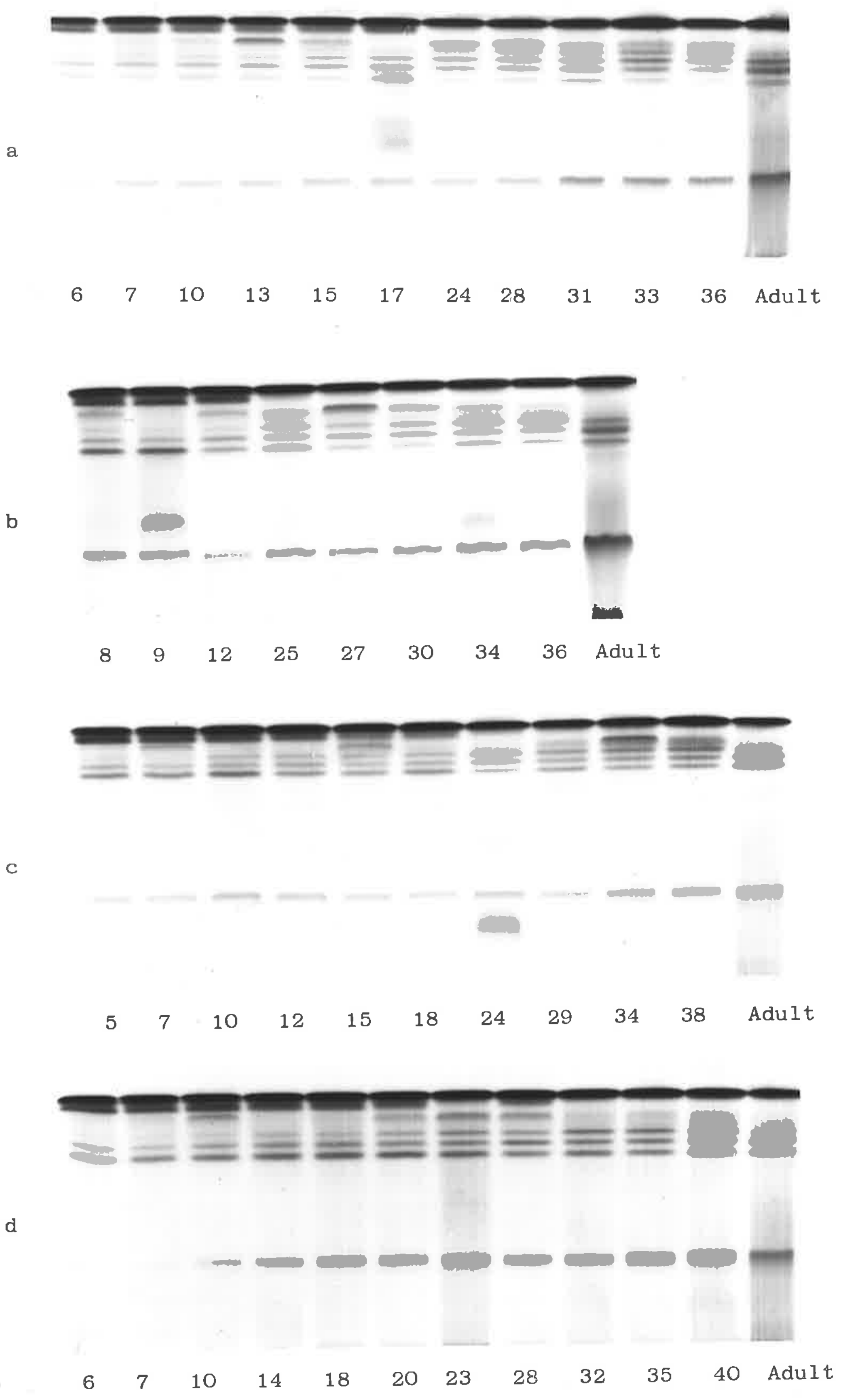

a
6 7 10 13 15 17 24 28 31 33 36 Adult
b
8 9 12 25 27 30 34 36 Adult
c
5 7 10 12 15 18 24 29 34 38 Adult
d
6 7 10 14 18 20 23 28 32 35 40 Adult

FIGURE 11.

Serum transferrin patterns of foetuses and adults of transferrin types

a. D_1E (samples 1-5) and

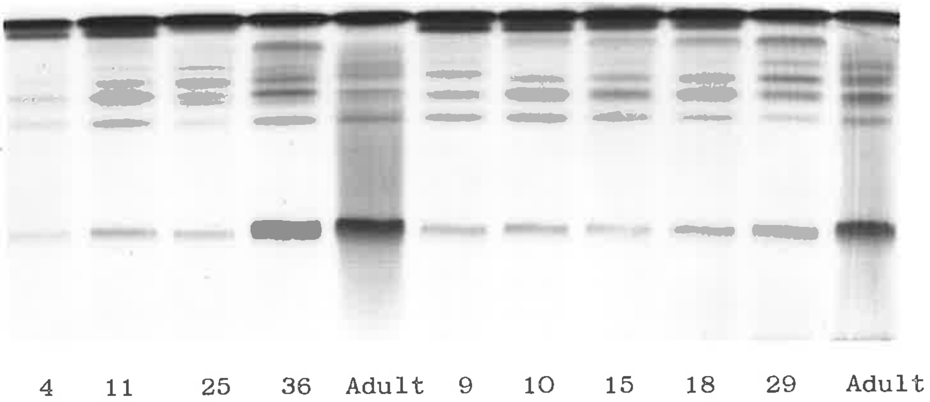
D_2E (samples 6-11)

b. AE

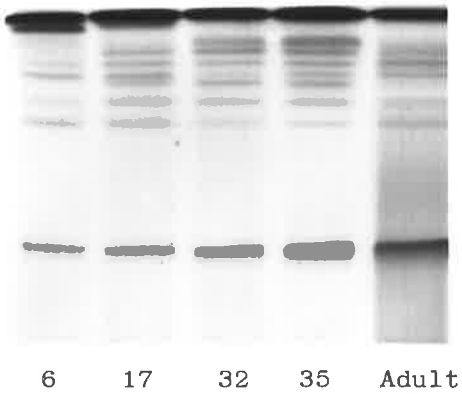
in tris-cacodylate

Sample	Length of foetus (ins.)	
	a.	b.
1	4½	6½
2	11½	17
3	25	32
4	36	35
5	Adult	Adult
6	9	
7	10½	
8	15	
9	18	
10	29	
11	Adult	

a



b



where the slowest zone is the major one, towards the adult type where the two slowest zones are of equal intensity, is actually carried further than this in older foetuses to give a pattern where the third slowest zone (zone 2) is the major one. The inferences that one can make on the structure of transferrin from these observations are given on page 69.

(ii) The J and Oc substances

The frequency of the four JOc inhibition types in the cattle foetuses have previously been presented in Table 23. There is an outstanding difference between the frequency of the inhibition types in the dams and the foetuses. The number of foetuses in the four inhibition classes in each length class are given in Figure 12. Although there is an apparent trend towards a reduction in the number of Oc foetuses with increased foetal length, there is no compensating increase in any other class, and from similar trends in all classes and in the foetuses not classified into inhibition type it may be concluded that there was some selection of younger foetuses for the sample. This is also indicated by Figure 13 where the inhibition types of the dams are graphed in a similar manner.

The titre of J and Oc substances in the sera of JOc foetuses is plotted against the length of the foetuses in Figure 14. There is no relationship between foetal length and the $\log_2$ titre of J and Oc substances.

In Figure 15 the titre of Oc substance in the sera of Oc foetuses is shown. There is a significant correlation between

FIGURE 12.

The frequency distribution of foetuses according to their crown-rump length and their J-Oc inhibition type.

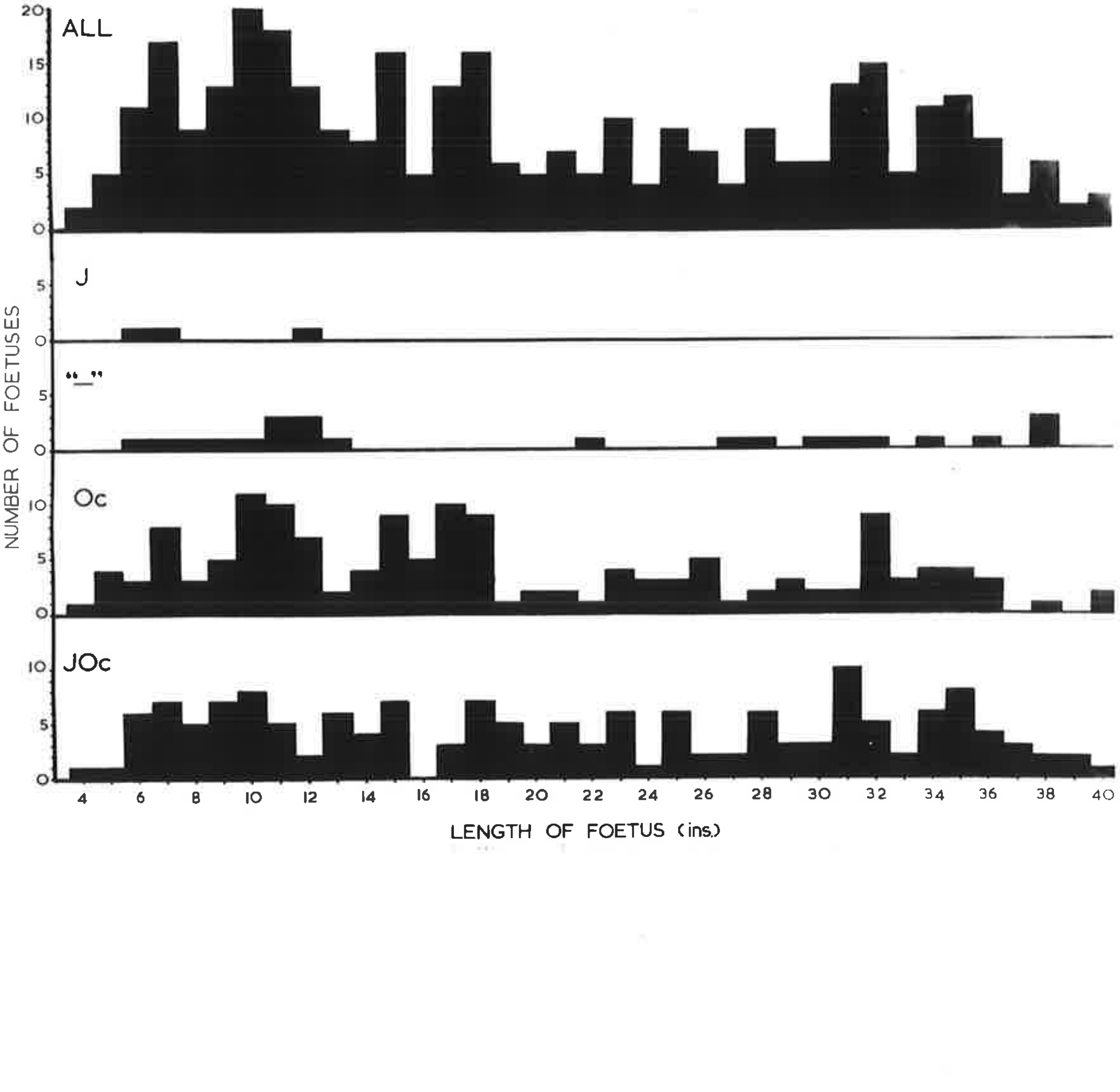

ALL
J
"–"
Oc
JOc
NUMBER OF FOETUSES
0
5
10
15
20
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
LENGTH OF FOETUS (ins.)

FIGURE 13.

The frequency distribution of dams according to their J-Oc inhibition type and the crown-rump length of the foetus they carried.

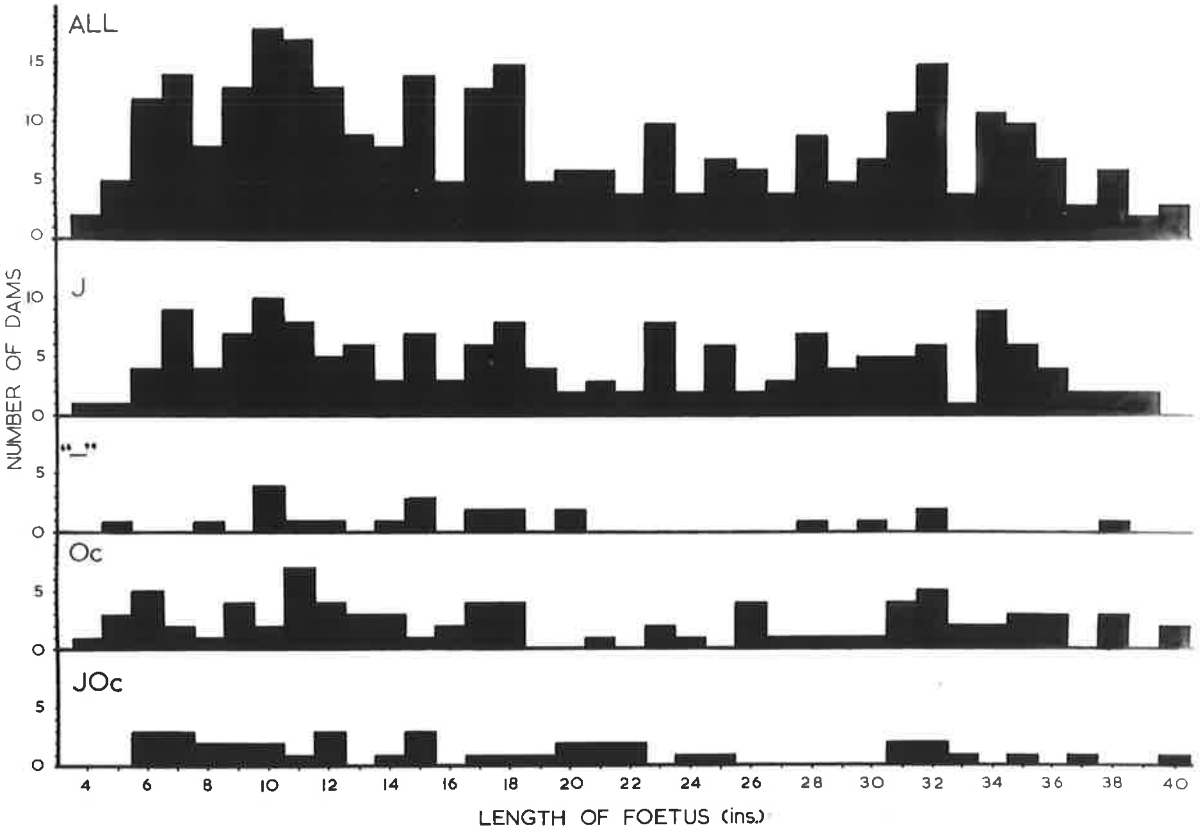
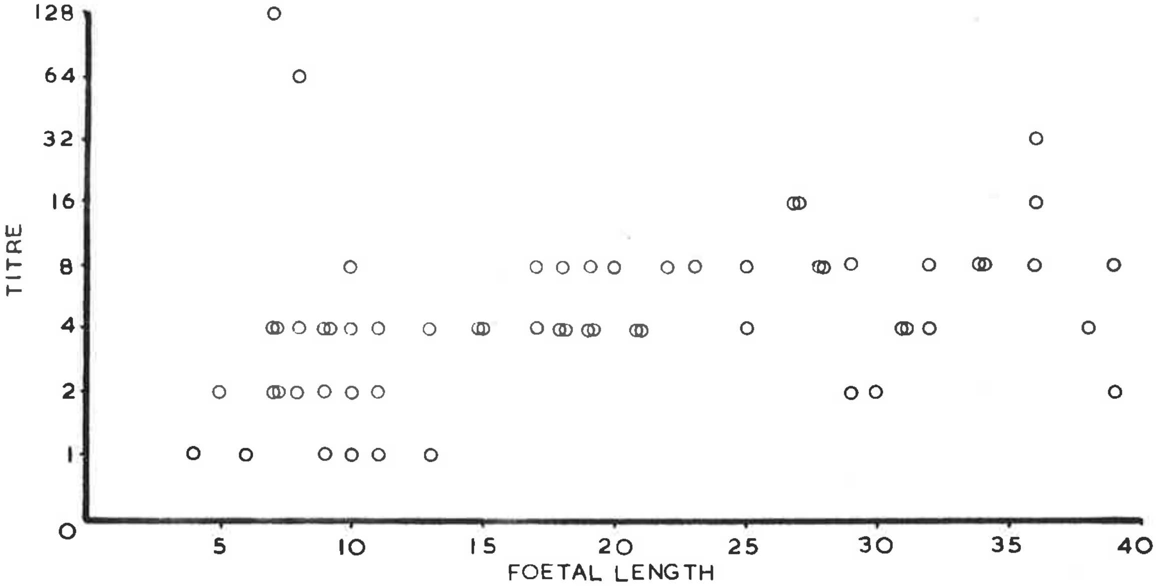

ALL
J
"–"
Oc
JOc
NUMBER OF DAMS
0
5
10
15
LENGTH OF FOETUS (ins.)
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40

FIGURE 14.

The titre of Oc and J substances in the serum of a sample of foetuses of inhibition type J-Oc and of various crown-rump lengths. The three closed triangles represent the three foetuses with only J substance in their serum. The titre scales are $\log_2$.

Oc SUBSTANCE



J SUBSTANCE

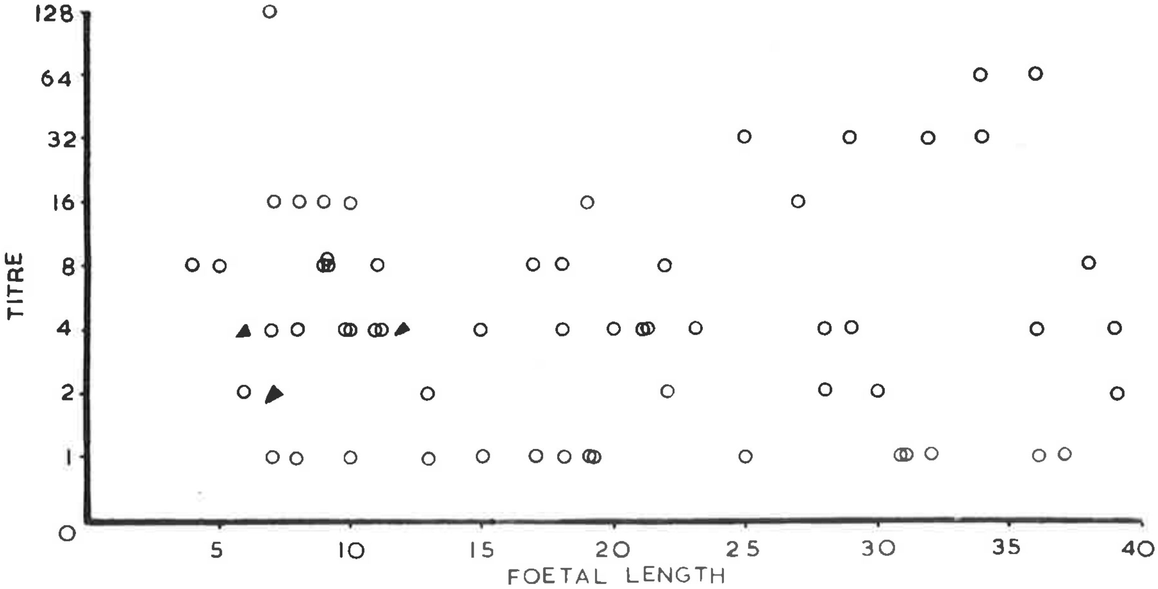
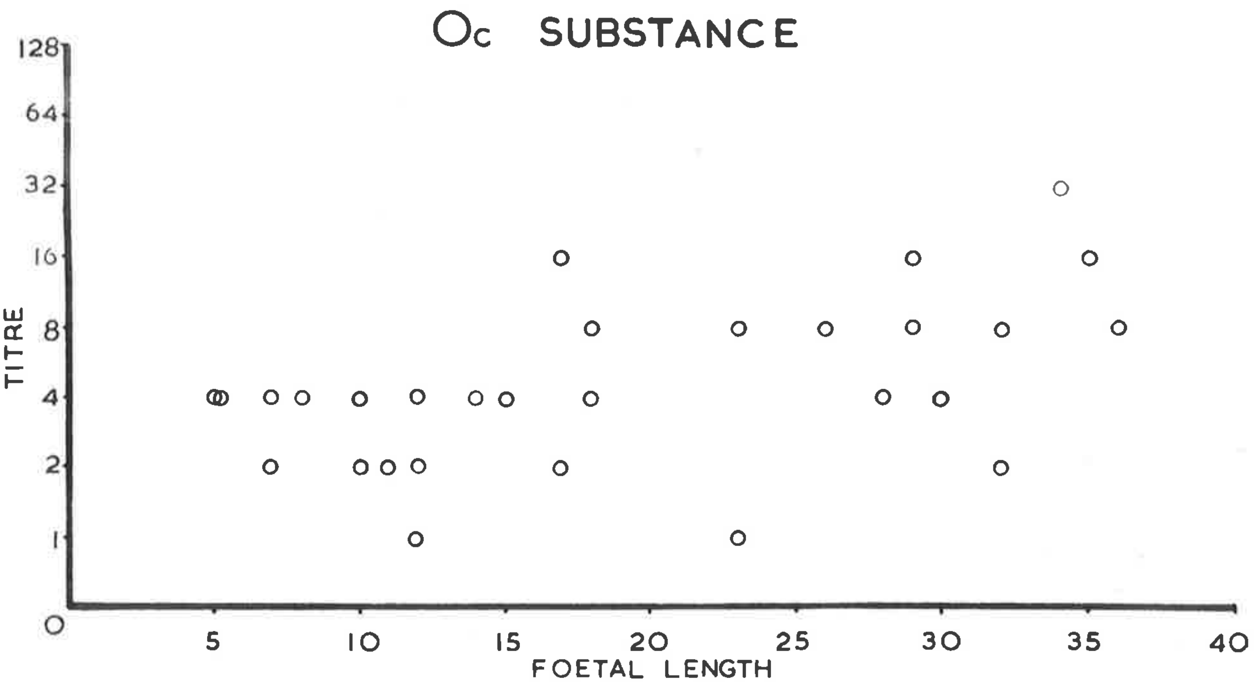


FIGURE 15.

The titre of Oc substances in the serum of a sample of foetuses of inhibition type Oc. The correlation coefficient relating $\log_2$ titre to foetal length is 0.516 ($.001 < P < .01$) in this sample.

? Is this right


O_C SUBSTANCE
TITRE
128
64
32
16
8
4
2
1
0
5
10
15
20
25
30
35
40
FOETAL LENGTH

titre and foetal length.

There is also a significant association between the titre of Oc and J substances in the sera of JOc foetuses (Figure 16). However, if the three sera showing the highest Oc titre are omitted from the analysis the correlation is not significant. These observations on the JOc blood-groups of foetuses are discussed in relation to the genetic control of the JOc blood-group system on page 72.

Table 26 shows the JOc inhibition types of foetuses and their dams. Although the data is unsuitable for incomplete family data analysis due to the dominance relationships between the alleles, it does provide information on possible transfer of blood group substances from dam to foetus. The table indicates that foetuses can have different inhibition types from their dams. This is a different finding to that in sheep (Appendix II) and is discussed on page 77.

(iii) Haemolysins and alkaline phosphatase

None of the foetal sera was shown to contain specific or non-specific haemolysins for sheep R and O red cells.

Cattle foetal sera showed the slow B phosphatase zone with mobility the same as that of sheep foetuses (Appendix II). The significance of this observation in relation to the failure to demonstrate an association between the JOc blood group and the A-O phosphatase polymorphism is discussed below (p. 68).

FIGURE 16.

The relationship between the titre of J and Oc substances in the sera of J-Oc foetuses, $r = 0.442$ ($.001 < P < .01$). If the three sera showing the highest Oc titre are omitted from the analysis, $r = 0.11$ which is not significant.

O_c & J SUBSTANCES

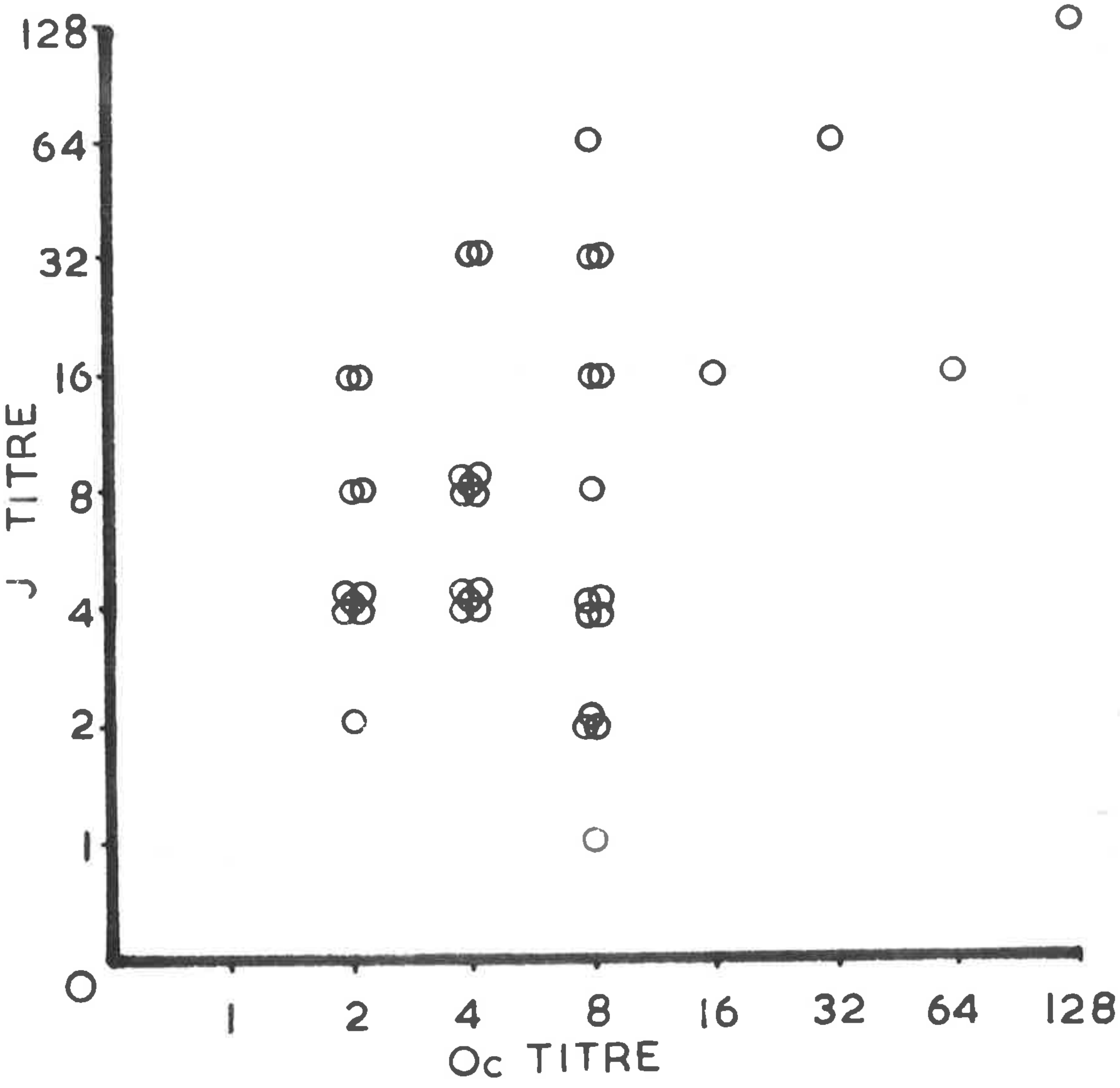


TABLE 26

THE J-Oc INHIBITION TYPES OF FOETUSES AND THEIR DAMS

INHIBITION TYPE OF DAM		INHIBITION TYPE OF FOETUS				TOTAL
		JOc	J	Oc	"-"	
	JOc	23	0	12	3	38
	J	107	3	43	14	167
	Oc	14	0	67	1	82
	"-"	4	0	16	3	23
	Total	**148**	**3**	**138**	**21**	**310**

2. Post-natal studies on blood group substances and transferrin

(i) Transferrin

The observation that all members of twin foetal pairs had the same transferrin and JOc type (Table 10, p. 45) was explained by the observation that all twins showed obvious venous anastomosis in utero. It should be possible therefore, to demonstrate transferrin mosaicism in new-born dizygous twins of different transferrin genotype. Two sets of twins and a set of triplets were examined and it was found possible to distinguish differences between transferrin patterns at 30 hours of age although the "foreign" transferrin persisted in twins for at least 7 days (Figure 17). Samples obtained from dizygous twin calves 30 minutes after birth, which were later shown to have different transferrin types, were indistinguishable. The significance of these observations is discussed below (p. 77).

Very interesting

(ii) J and Oc substances

From the high proportion of foetuses with Oc substance in their serum compared with the population of dams from which they were taken, it was inferred that some change in JOc phenotype took place after birth. A series of samples was taken from calves beginning as soon after birth as possible. The results of JOc typing are given in Table 27. The changes in phenotype observed are probably not due to absorption of serum antigen by ingested antibody (via colostrum) since haemolysins were detected in calf serum at the first sampling which was usually within 12

FIGURE 17.

The transferrin patterns of a pair of dizygous twins sampled 30 hours, 2, 3, 4, and 5 days after birth.

Citrate buffer

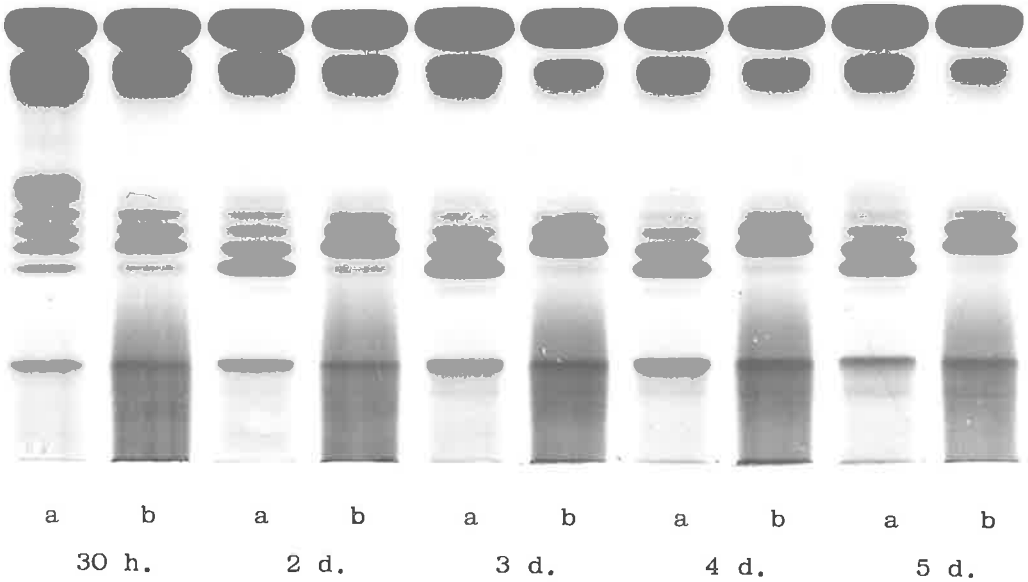

a
b
a
b
a
b
a
b
a
b
30 h.
2 d.
3 d.
4 d.
5 d.

TABLE 27

THE J-Oc PHENOTYPES OF CALVES SAMPLED AT INTERVALS AFTER BIRTH

CALF NO.	AGE (DAYS)					
	BIRTH⌿	3	7	10	14	115
1	JOc	Oc	Oc	"-"	"-"	Oc
2	Oc	Oc	Oc	Oc	Oc	N.T.*
3	Oc	Oc	Oc	Oc	"-"	Oc
4	Oc	Oc	Oc	"-"	N.T.	Oc
5	J	"-"	"-"	"-"	"-"	N.T.*
6	JOc	JOc	JOc	J	J	N.T.*
7	JOc	JOc	N.T.	N.T.	N.T.	J
8	JOc	JOc	N.T.	N.T.	N.T.	"-"

⌿ The first sample was taken when new-born calves were first discovered on the day of birth.

N.T. = not tested (died*)

hours of birth. Only the observations on calves 6 and 7 are consistent with the change expected from observations on the low frequency of the "J" phenotype in foetus. A more interesting observation that can be made on Table 27 is that the Oc blood-group substance can disappear from the sera of an individual and reappear sometime later. Whether this is only found in growing calves is not known, however the observation does indicate that the Oc substance of adult cattle sera could be re-examined with the view to determining whether or not the phenotype is constant throughout the life of an animal. Sprague (1958a) did not record having examined the same individuals at different stages of development. Observations on a single sample from the standard J cow (NF 68) indicated that the titre of J substance on red cells at least can vary with physiological conditions since a short time after calving the cow's red cells could be haemolysed by half the amount of antibody normally required.

d. Phylogenetic studies on cattle species

1. Bibos banteng

Figure 18 is a photograph of the South-East Asian banteng cattle. The male is black and the female brown. This is a sexual dimorphism for the presence and absence of hair, both sexes having black skin. The shoulder hump is due to the dorsal extension of the spinous processes of the thoracic vertebrae and is not comparable with the fatty hump of the zebu breeds.

The transferrin patterns of some of these animals are shown in Figure 19(a) together with some cattle transferrin standards. All 30

FIGURE 18.

Photographs of the Banteng cattle of South-East Asia.

TOP	Female
BOTTOM	Male

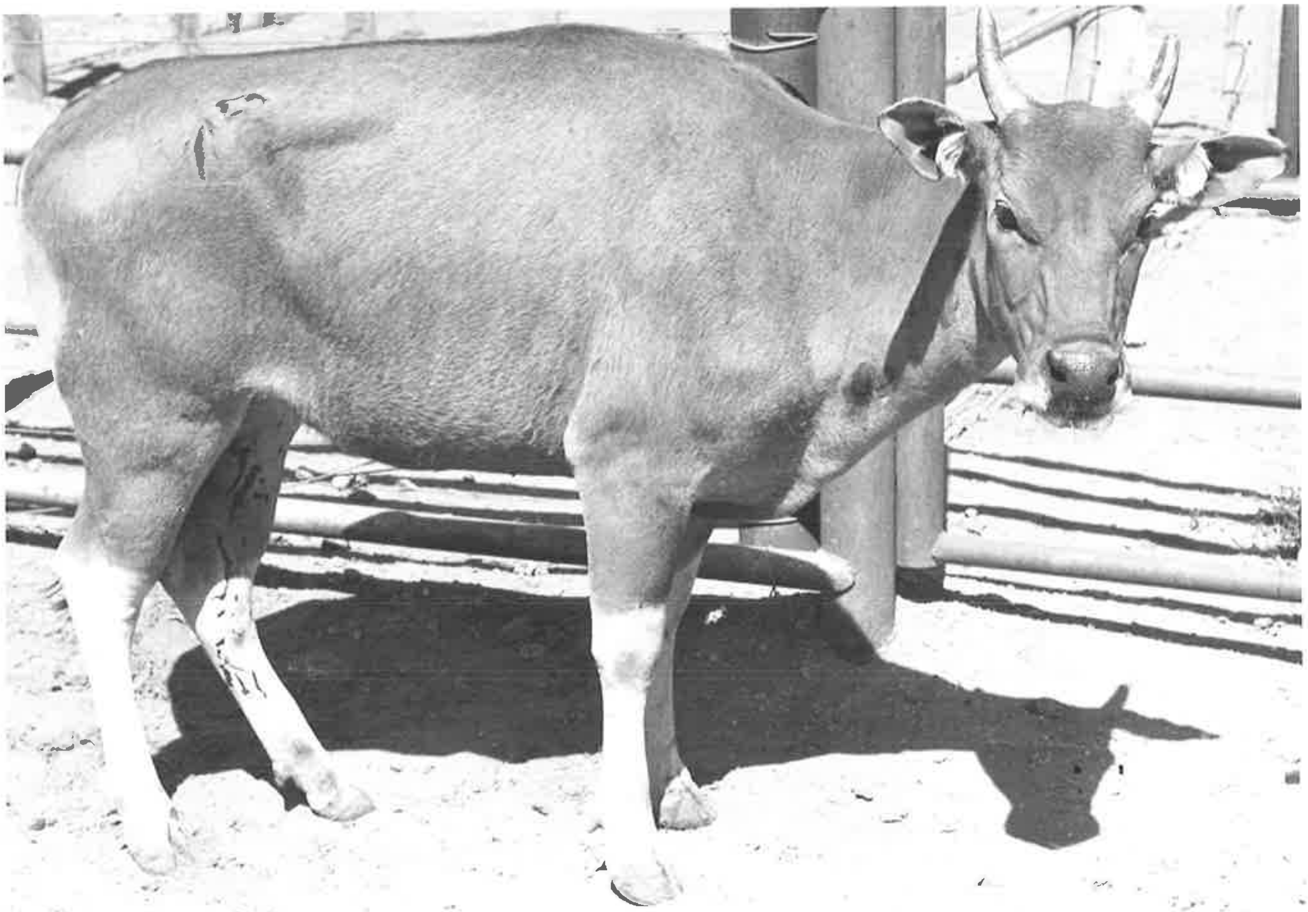


FIGURE 19.

a. Serum transferrins of Bibos banteng compared with domestic cattle standards.

Samples 1, 11 and 12 are cattle standards and all others are from banteng cattle. All banteng samples are transferrin type D_1.

Sample 5 which might be typed as D_1D_2 was confirmed as D_1 on re-running.

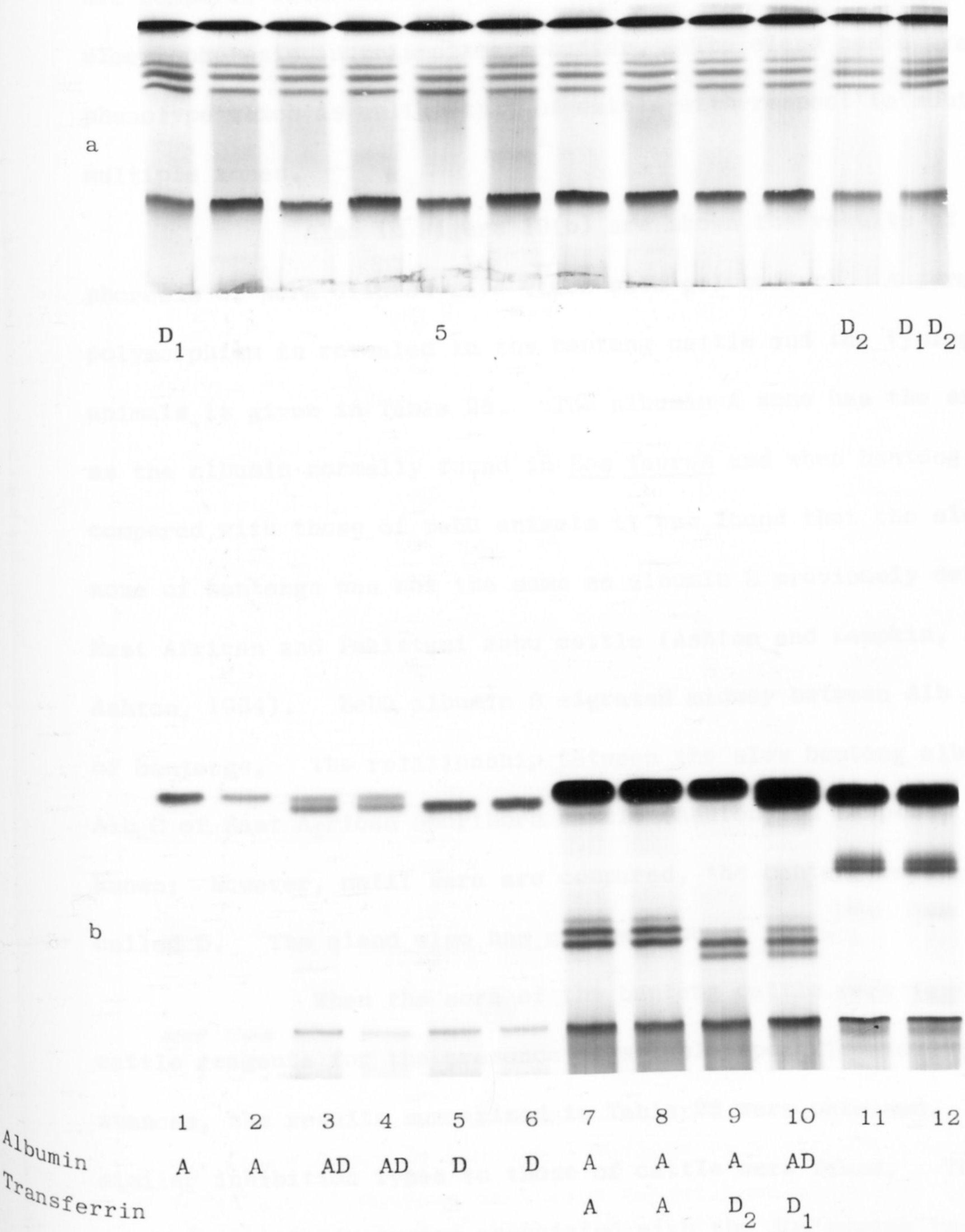
b. Transferrins and albumins of Bos taurus, Bibos banteng and Taurotragus oryx (eland).

Samples 1-6 diluted 5 times.

Samples 1, 2, 7, 8 and 9 are Bos taurus sera.

Samples 3, 4, 5, 6 and 10 are Bibos banteng sera.

Samples 11 and 12 are replicates of an eland sample.

a
D1
5
D2
D1D2
b
1
2
3
4
5
6
7
8
9
10
11
12
Albumin
A
A
AD
AD
D
D
A
A
A
AD
Transferrin
A
A
D2
D1

banteng sera examined showed a transferrin pattern which was indistinguishable from cattle TfD_1 (Table 28). In Figure 19(b), banteng transferrins are compared with those of cattle and the eland (Taurotragus oryx) by gel electrophoresis in tris-citrate buffer. The eland has a transferrin phenotype which is unlike that of cattle with respect to mobility or multiple zones.

[illegible]

Also in Figure 19(b) are shown the results of electrophoresis of sera diluted five times with gel buffer. A serum albumin polymorphism is revealed in the banteng cattle and the typing of 15 animals is given in Table 28. The albumin A zone has the same mobility as the albumin normally found in Bos taurus and when banteng albumins were compared with those of zebu animals it was found that the slow albumin zone of bantengs was not the same as albumin B previously described in East African and Pakistani zebu cattle (Ashton and Lampkin, 1965a; Ashton, 1964). Zebu albumin B migrated midway between Alb A and Alb D of bantengs. The relationship between the slow banteng albumin and Alb C of East African Shorthorn cattle (Ashton and Lampkin, 1965a) is not known; however, until sera are compared, the banteng variant will be called D. The eland also has a slow albumin zone.

When the sera of the banteng cattle were tested with cattle reagents for the presence of soluble specific blood-group substances, the results summarized in Table 28 were obtained. That is, similar inhibition types to those of cattle were found. The naturally occurring antibody system associated with the JOc system is somewhat

Sprague had
anti-O in [illegible]
" —/— " [illegible]
only!

TABLE 28

TRANSFERRIN, ALBUMIN AND J INHIBITION TYPES OF 15 BANTENG CATTLE AND SEVEN ASIATIC BUFFALO

Species	No.	Transferrin≠	Serum≠ Albumin	J Inhibition*	Antibody And Titre	
Bibos banteng	159	D_1	D	J	O	8
	166	D_1	D	J	O	32
	158	D_1	D	JOc	-	
	1002	D_1	D	J	O	8
	171	D_1	AD	JOc	-	
	1122	D_1	D	JOc	-	
	169	D_1	AD	J	O	32
	468	D_1	AD	J	O	32
	170	D_1	D	JOc	-	
	164	D_1	D	JOc	-	
	466	D_1	D	JOc	-	
	1118	D_1	D	JOc	-	
	454	D_1	D	JOc	-	
	MZ1	D_1	AD	J	O	16
	150	D_1	AD	JOc	-	
Bubalus bubalis	B1	B		JOc	-	
	B3	A		JOc	-	
	1217	B		J	-	
	1208	AB		J	-	
	1215	AB		J	-	
	1202	AB		J	-	
	1209	AB		JOc	-	

* All Banteng cattle had the J antigen on their red cells.

≠ A further 15 Banteng sera were recently examined and all were transferrin type D . Nine animals were albumin type D, 4 AD and 2 A.

different from that described in Bos taurus. In every serum where Oc substance was absent, the anti-sheep O antibody was present. No anti-J was found (Table 28). fine

2. Bubalus bubalis

In Figure 20(a) the transferrins of the Asiatic buffalo are compared with those of cattle. There is no cattle mobility phenotype that is the same as the buffalo phenotypes. Buffalo A is faster than cattle A, and buffalo B is faster than cattle B. The result of electrophoresis of buffalo serum in tris-cacodylate buffer is shown in Figure 20(b). It is apparent that the multiple zone phenotype (without regard to their mobility) of buffalo B is the same as that of cattle D_1 and D_2, the second slowest zone being resolved into two zones. Table 28 gives the transferrin type of the eight buffaloes studied together with their JOc inhibition type. No sera contained haemolysins against sheep R or O red cells (Table 28).

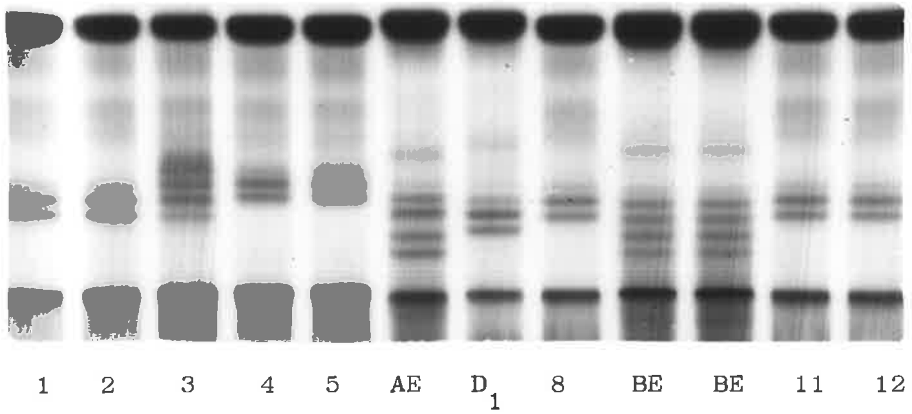
These observations on the serum proteins and blood-group substances in banteng cattle and the buffalo are discussed in relation to their taxonomy on page 79.

FIGURE 20.

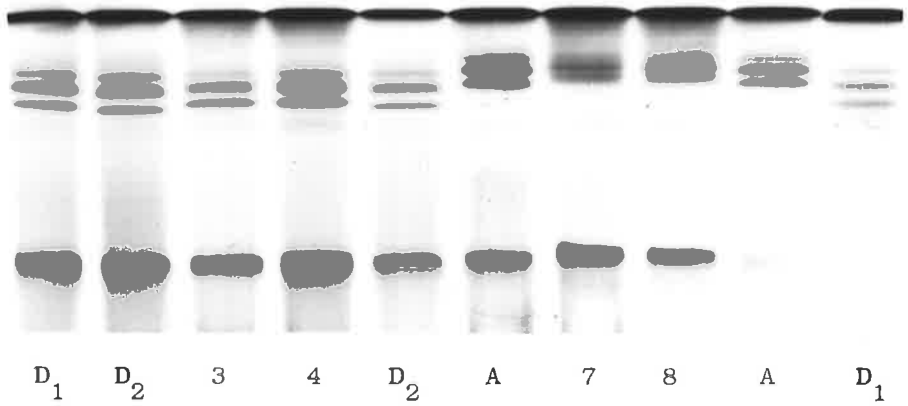
a. Serum transferrin patterns of the Asiatic buffalo compared with those of cattle in tris-citrate buffer.
Samples 1 and 2 are the slow buffalo variant (B), samples 4 and 5 are the fast variant (A), and sample 3 is the third type of pattern found (AB).
Samples 8, 11 and 12 are also the slow variant (B).
Samples 6, 7, 9 and 10 are cattle standards.

b. Serum transferrin patterns of the buffalo compared with those of cattle in tris-cacodylate buffer.
Samples 3, 4, 7 and 8 are buffalo samples. Note the split zones of the slower variant.
Samples 1, 2, 5, 6, 9 and 10 are cattle standards.

a



b



IV. DISCUSSION

a. The multiple allele hypothesis for the inheritance of transferrin patterns in cattle

Most data presented in the literature are in agreement with the hypothesis that the variable transferrin patterns observed in cattle are due to the segregation of a number of alleles at a single transferrin locus. Exceptions to this hypothesis have been reported (Smithies and Hickman, 1958; Gahne, 1961) though these have been attributed to errors in the parentage records. The paucity of exceptions to the allelic hypothesis in the literature is of interest since in the author's experience the frequency of misclassification of calves in stud herds at least is appreciable. Early data published by Ashton (1957), before the multiple allele hypothesis was proposed, can now be seen to contain 8 exceptions in 150 matings.

The dam-foetus pairs examined revealed no exceptions to the allelic hypothesis for the inheritance of transferrin patterns in cattle. It is probable therefore, that the exceptions to this hypothesis that have been reported in the literature are in fact due to errors in parentage records, the explanation usually invoked.

b. Segregation ratios at the transferrin locus

Using the method of Cooper (1966) to analyse the dam-foetus family data for the expected segregation of transferrin alleles, no evidence was found to suggest that in this sample there is any excess of heterozygous offspring over that expected, assuming that heterozygotes and homozygotes are expected with equal frequency from heterozygous dams (Table 8, p. 42). Thus, the indirect evidence obtained by Ashton and Fallon (1962) that heterozygotes are more viable _in utero_ is not confirmed by this study of foetuses. It could be argued that the viability effects may not have had time to manifest themselves in the foetuses studied; however, the criterion for the death of a conceptus by the above workers was a return-to-service after 25 days or more. The youngest foetus obtained in the abattoirs study was 75 days old (4½ inches crown-rump length; Lyne, 1960), so that any effect of transferrin type on foetal viability as envisaged by Ashton and Fallon (1962) should be detectable by a study of foetal transferrin types. The data of Ashton and Fallon (1962) are based on the records of A.I. centres which are compiled only on the basis of farmer requests for a return of service. In these author's own words, "Clearly, returns-to-service data must be interpreted with caution."

Although the New Zealand work (N.Z. D.P.M.B., 1963) showed results similar to the Queensland study (though non-significant) the dam-foetus data presented here actually show a non-significant excess of homozygotes.

There is a non-significant deficiency in the expected proportion of the offspring of $\underline{Tf}^{A}/\underline{Tf}^{D1}$ dams with the $\underline{Tf}^{D1}$ allele. Whilst this could indicate a disturbed segregation of $\underline{Tf}^{A}$ and $\underline{Tf}^{D_1}$ alleles, other heterozygous dams carrying $\underline{Tf}^{D_1}$ actually show a non-significant excess of offspring with $\underline{Tf}^{D_1}$. The data therefore show no consistent evidence for disturbed segregation ratios at the transferrin locus.

Although the number of twin and dead foetuses found in this study was small, the material is potentially suitable for a study of the effect of the dam's transferrin type on her fertility as judged by the frequency of twinning and foetal mortality. No conclusions can be drawn from the results set out in Table 10 (p. 45) except those relating to transferrin mosaicism discussed below (p. 77).

The failure to demonstrate maternal-foetal incompatibility with respect to transferrin type (Table 9, p. 43) confirms the results of a number of workers (literature survey, p. 13). There is no evidence for antagonism between the transferrin types of dam and foetus when the foetuses are considered with or without regard to their stage of development. If the incompatibility effect proposed by Ashton (1959b) was operative early in foetal life, one might have expected that the older foetuses ($>$20") would show a greater tendency towards a deficiency of foetuses incompatible with the dam's transferrin type. In fact, there was a non-significant excess of older foetuses in the incompatible class. It is concluded that the population of animals sampled at the abattoirs show no transferrin maternal-foetal incompatibility effect.

None of the half-sib families showed significant departures from the expected 1:1 ratio for heterozygotes:homozygotes that could not be explained by chance alone. Two of the three significant departures from expectation (Table 12, p. 46) were in favour of homozygotes whilst the third showed an excess of heterozygotes. These observations are compatible with those made on the abattoirs material. However, two reservations must qualify the failure of these observations to demonstrate an excess of heterozygotes over homozygotes in the offspring of heterozygous sires: firstly, since the terms heterozygous and homozygous are no longer meaningful with respect to $\underline{Tf}^D$, any conclusions drawn from the observations are of doubtful value. Secondly, since it was demonstrated that the analysis of half-sib family data using the sire's inferred transferrin type was probably invalid due to errors in parentage records, the essentially negative finding must be considered with this in mind.

c. A possible second transferrin locus in cattle

The observation that some cattle can inherit a slow transferrin zone seemingly independent of the inheritance of the regular variants (p. 48) leads to the conclusion that individuals with the additional zone have at least one additional gene concerned either with the production of multiple zones from the existing transferrin polypeptides or with the synthesis of a new transferrin polypeptide. The examination of a large number of autoradiographs reveals that most cattle have some iron-binding protein in the region of the H zone and that there is variable expression of this between individuals, from absent to strong (as in the case of bull X, Figure 7, p. 48). The likely explanation for the inheritance of the H zone is therefore the first suggestion above, namely a gene or genes controlling the degree of formation of the H zone in the multiple zone system by polymerization, carbohydrate addition or other means. The variable possible but not very likely

intensity of the H zone between individuals could be due to the segregation of a number of alleles at a locus.

Whether the gene or genes postulated is at the same locus as the gene involved in the formation of the regular multiple zones by the addition of carbohydrate (Chen and Sutton, 1967) is unknown; however, this is the simplest hypothesis in the absence of further evidence.

Variation within individuals has not been thoroughly investigated. However, two samples taken from bull X two years apart had indistinguishable transferrin patterns.

d. Transferrin population studies

The collection of two successive generations in the form of dam-foetus pairs provides ideal material in which to study possible changes in genotypic and gene frequencies from one generation to the next. It also allows examination of foetuses of various ages to determine whether the gene and genotypic frequencies alter during intrauterine life, assuming that the old and young foetuses both represent unbiased samples of the same large foetal population.

No significant differences were found in gene and genotypic frequences from one generation to the next or during foetal life (Tables 14, 15, 16, p. 47) and it is concluded that any selection favouring a gene or genotype is extremely mild.

Although other workers have found an excess of transferrin heterozygotes over that expected in cattle populations assuming random mating (p. 17), the results presented in Table 16 (p. 47) show no

agrees with Cooper Pendel

evidence for this. Since the abattoirs sample represents a better sample of a given large interbreeding population than does any sampling involving herds of related animals, the present results are good evidence against strong heterozygote superiority as a major factor maintaining the transferrin polymorphism. As pointed out by Robertson (1965), sampling of herds involving few sires can lead to an apparent excess of heterozygotes in the sample since the expected proportion of heterozygotes is underestimated. Jamieson's (1965) observation that the degree of heterozygote excess was dependent upon the herd examined may be related to the Robertson effect or it may be the result of an effect noted by Cooper (1966) where heterozygote excess was only detected in inbred populations of Merino sheep. The Robertson effect was not noted in the dairy-farm half-sib-family samples reported in this thesis (p. 48). Even in the Friesian sample where only 15 sires contributed to the population in 8 herds, the observed and expected population genotypic frequencies were almost identical (Table 18, p. 48). Although gene frequencies were similar to Ashton and Fallon's (1962) reported frequencies ($\underline{Tf}^{D}$ not being subdivided) the excess of heterozygotes reported by them was not found in the dairy cattle population. The fact that these workers could only find such an effect in one out of four populations suggests that it is dependent upon the background genotype of the animals or due to an artifact induced by the sampling method, most likely the Robertson effect.

e. Breed differences in the distribution of transferrin and J alleles in cattle

Jamieson (1966) has presented the results of a very extensive survey of transferrin genes among cattle breeds in the U.K. and other parts of the world. A problem with breed comparisons is the definition of the term "breed" itself. A number of the studies reported on transferrin gene frequencies in different breeds are actually studies of nominal breed classifications only, e.g. Ashton (1961), Ashton and Fallon (1962). Even within purebred registered cattle, types have arisen that are characteristic of regions and countries. Thus "breed differences" should be considered with caution and regarded as population differences. The most widely documented and acknowledged "breed difference" is the frequency of $\underline{Tf}^E$ in different populations. Ashton (1958d) postulated that the relatively high frequency of $\underline{Tf}^E$ in northern U.K. cattle and in zebu cattle was due to some climate tolerance conferred on an animal by this gene. Osterhoff (1966a) could find no differential effect of extreme climate stress on cattle with and without $\underline{Tf}^E$ and so the "climate tolerance" conferred by this gene remains a matter of surmise. Although in the South Australian sample the frequency of $\underline{Tf}^E$ in purebred Friesian cattle was 10 times that in purebred Jersey cattle, the allele was not "absent from Jerseys" as claimed by Ashton (1958d). The allele was also found in Jerseys by C. Stormont (Jamieson, 1966). Another explanation for a gradation in the frequency of an allele from one geographic location to another is the diminishing or increasing influence of one initially isolated population on another. This seems to be the case with haemoglobin B where it is postulated that the presence of the $\underline{Hb}^{B}$ allele in some European cattle breeds (e.g. Jersey) is evidence for zebu introduction into these areas in ancient times (Boston, 1954; Bangham and Blumberg, 1958). Table 29 demonstrates the high frequency of $\underline{Hb}^{B}$ in a Jersey cattle population and its very low frequency in a Friesian population and is in agreement with the results of Bangham (1957). The hypothesis for the origin of the $\underline{Hb}^{B}$ allele in Jersey cattle is not compatible with the observed frequency of $\underline{Tf}^{E}$ in this breed if it is postulated that $\underline{Tf}^{E}$ in European cattle also originated from zebu breeds. This argument is of course extremely weak in the absence of information on the selective forces associated with the maintenance of the haemoglobin and transferrin polymorphisms in different populations. One might infer that the $\underline{Hb}^{B}$ allele is maintained in the Jersey breed by selective forces that are not present in the Friesian breed since the Jersey breed is effectively closed whilst Friesian animals may be graded up from non-purebred stock by persistent mating with purebred animals (Jersey and Friesian Herd Books, 1965, 1966). It is surprising therefore, that the $\underline{Hb}^{B}$ gene does not find its way into Friesian populations.

not so surprising!

In contrast with the marked variation in transferrin gene frequencies between cattle populations, the frequency of the J inhibition types is remarkably constant from one population to the next. The results presented in Table 22 (p. 49) support this, the frequency of J inhibition types in the sample of purebred Jersey and Friesian

TABLE 29

THE FREQUENCY OF HAEMOGLOBIN PHENOTYPES IN PUREBRED JERSEY AND FRIESIAN CATTLE IN SOUTH AUSTRALIA

	Jersey (11 herds)		Friesian (9 herds)	
	No.	Frequency	No.	Frequency
Hb-A	15	0.24	26	1.00
Hb-AB	31	0.50	0	0.00
Hb-B	16	0.26	0	0.00
Total	62		26	

samples being very similar. This constancy of the proportion of animals with J substance in their sera between populations may be a consequence of the number of alleles determining the presence of J substance in the serum (at least 8). In the presence of such a large number of alleles the frequency of any one could vary considerably between populations whilst the phenotypic frequency would be relatively insensitive to these fluctuations. When the J-Oc inhibition types are considered however, populations show considerable variation in phenotypic frequency. Sprague's (1958a) three populations were markedly different from each other and from the sample of cattle collected at the abattoirs in South Australia (Table 23, p. 50). Thus, with respect to the J-Oc blood-group system as a whole, the frequency of inhibition types is not constant between breeds and populations of cattle and there are no apparent patterns.

f. The association of serum alkaline phosphatase type with the JOc blood group system

The failure to find an association between the presence of Oc blood-group substance and an alkaline phosphatase isozyme in the serum of cattle sampled at an abattoirs (p. 51) is compatible with Gahne's (1936b) proposal that the A phosphatase zone in cattle shows simple Mendelian inheritance. That is, assuming that the loci controlling Oc substance and alkaline phosphatase synthesis are not closely linked, one would not expect to find an association between the phenotypes. It is surprising therefore, that Rendel and Gahne (1963) and

Rendel and Suzuki (1964) found an association between J blood-group type and the presence of the A phosphatase isozyme. That is, for the genetic studies on alkaline phosphatase variants and those on the J-Oc blood-groups to be consistent with one another one would expect either no association or complete association between the blood-group and isozyme phenotypes for the cases of independent segregation and pleiotropy with complete penetrance respectively. It would not be possible of course, to distinguish easily between pleiotropy and very close linkage, given linkage disequilibrium.

It is possible that the genetic work actually involved a different isozyme to the correlation work due to different staining conditions although the method quoted was the same. In view of the observation that some cattle sera have a slow alkaline phosphatase isozyme (Gahne, 1963b), as do cattle foetuses (p. 55) and that this has about the same mobility as that of sheep where activity is almost completely dependent upon the presence of O substance in the serum, it is possible that this enzyme could be more strongly associated with the presence of a blood-group substance. Unfortunately sera were not scored for this zone in this study.

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g. Blood-group substances and transferrin in foetal serum

1. Inferences on the structure of transferrin

For a given transferrin genotype, the transferrin pattern is variable depending upon foetal age (p. 53). Similar observations have also been made on rats (Wise *et al.*, 1963). In man, though the adult phenotype is characterized by a single strong zone, foetal serum has four additional slower transferrin components (Parker et al., 1963; Melartin et al., 1966) and it has been postulated that the 5 zone pattern is the result of a deficiency in the enzyme attaching sialic acid side chains to the protein. The slowest zone would therefore represent transferrin with no carbohydrate moiety attached, and the fastest (equivalent to that of adults) would represent transferrin with the full complement of sialic acid residues.

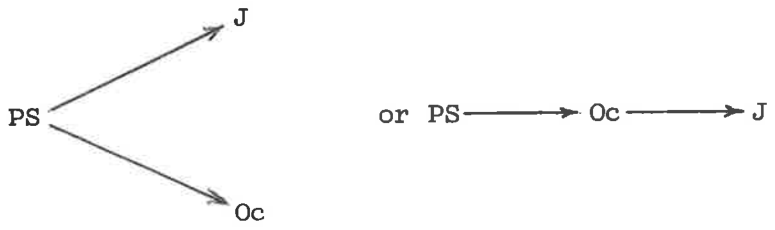
The five zones of human foetal transferrin may be compared with the five zones of adult cattle homozygotes. If multiple transferrins in cattle are due to the variable addition of carbohydrate to the protein, then for a negatively charged carbohydrate the fastest zone would have four residues and the slowest none. It would be necessary to postulate therefore, that in the normal adult the activity of the enzyme attaching carbohydrate to transferrin is below that required to saturate all transferrin molecules. In young foetuses the activity of the enzyme may be less than in adults since the predominant transferrin is the slowest. In older foetuses this slower zone is actually less intense than the second slowest and therefore the enzyme's activity would have to rise above that found in adults. There is some experimental evidence to support the carbohydrate theory and the observations on foetal transferrin patterns are at least consistent with it. The patterns might also be interpreted in the same sense but involving enzymically controlled multimer formation instead of carbohydrate addition. However, this would mean that the slowest zone would have five times the molecular weight of the fastest, given that the fastest represents the smallest unit possible. The differences in mobility of the members of the zone quintet are probably not compatible with this hypothesis, and Chen and Sutton (1967) could find no evidence for the presence of molecules with molecular weight differences of this order in ultracentrifuge studies.

2. Models for control of the JOc system

Although the correlation between J titre and foetal age proposed by Schmid and Buschmann (1962) from very limited data was not found (p. 54), a random sample of Oc foetuses showed a positive correlation between foetal length and Oc titre. This was not so when the Oc substance occurred with J substance, i.e. in JOc foetuses (Figure 14, p. 54). Thus it could be postulated that where there is no competition for a common precursor between the J forming system and the Oc forming system, the amount of soluble blood-group substance produced depends upon foetal age. Due to the fact that only three "J" foetuses were obtained, this hypothesis could not be tested with respect to the J system.

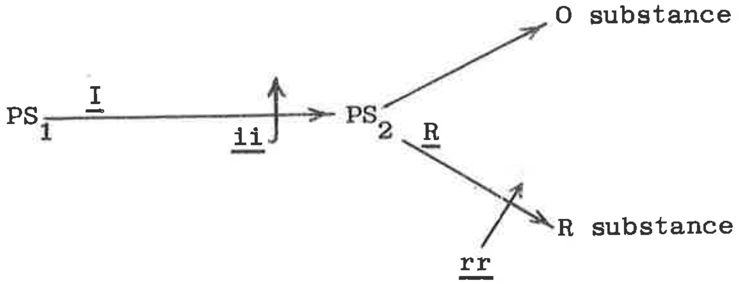
Rendel and Suzuki (1964) found a negative association between the titres of J and Oc substances in adult sera and they suggested that this indicated that the J and Oc substances had a common precursor substance (PS):

i.e.

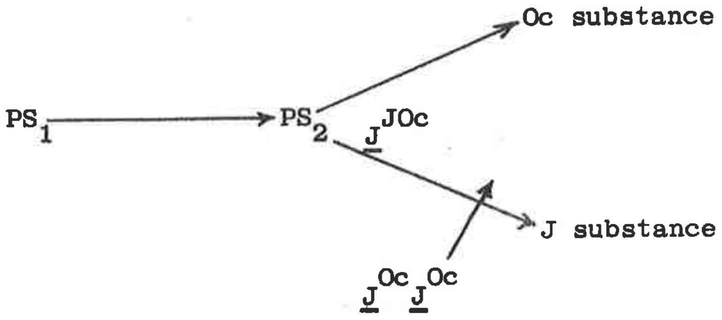


However, it is difficult to postulate mechanisms for these pathways that are consistent with the genetic hypothesis of Sprague (1958a), i.e. that the JOc blood-group system is under the control of four alleles at the J locus, viz. $\underline{J}^{J}$, $\underline{J}^{JOc}$, $\underline{J}^{Oc}$ and $\underline{j}$ (see Table 3, p. 20). There were, however, three exceptions to the hypothesis although the reason for two of these was given as a mixup in the samples.

The significant correlation between the titres of J and Oc substances in foetuses is a similar observation to that made on sheep foetuses of inhibition type R/O (Appendix II). The model proposed in Appendix II to explain the observations on the sheep R-O-i system is:



Assuming that the cattle system is basically similar to this, the following model might be postulated:



This is consistent with the observations on correlated titres in foetal serum and with the genetic evidence on the inheritance of the Oc and JOc phenotypes, i.e. the Oc phenotype is equivalent to the O of sheep and the JOc phenotype is equivalent to the R of sheep since R sheep are actually R/O. Observations on the J and "-" phenotypes are more difficult to incorporate into the same scheme since they have no genetic equivalent in the sheep R-O-i system, the phenotypes in cattle being determined by two additional alleles in the same series whilst the sheep "-" (i) is determined by genes at a second locus. Sprague (1958a) suggested that one of his exceptions to the multiple allelic hypothesis may have been due to the segregation of alleles at a second locus. Thus the exception to his hypothesis; Oc x JOc ⟶ "-", has an equivalent in sheep studies, viz. O x O ⟶ i. There are two observations that indicate that two loci may be involved in the control of the JOc blood group in cattle.

''-'' = i,

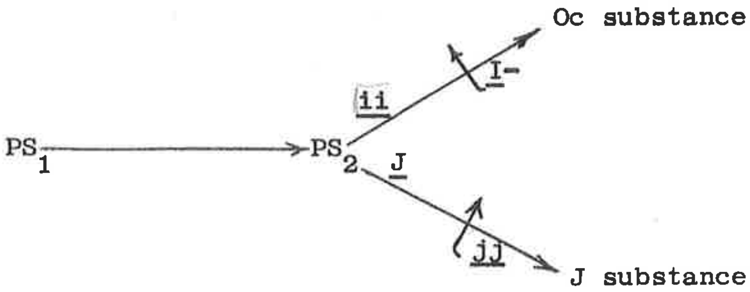
(i) The distributions of the four phenotypes in dams and foetuses are markedly different. It may be inferred that some of the foetuses of type Oc and JOc would become type J in adults. Since there is no tendency for an increase in the frequency of type J foetuses with increasing foetal age and a proportional decrease in the frequency of JOc and Oc types, any change that takes place must be post-natal, such a postulated change has been demonstrated in calves (Table 27, p. 56). It seems that some J and "-" adults have the genetic information for the synthesis of Oc substance but this is not expressed.

(ii) Table 25 (p. 52) shows the inhibition types of a number of bulls from which both blood serum and seminal plasma could be obtained. Different inhibition types are expressed depending upon which fluid is used as an inhibitant. This further supports the hypothesis that some individuals do not express their full genetic potential for blood group substance production.

Taking into account the comment previously made about Sprague's (1958a) model (p. 20) that it is curious that in the presence of $\underline{j}$, neither $\underline{J}^{Oc}$ nor $\underline{J}^{JOc}$ produce the Oc substance, the following genotype-phenotype relationships may be proposed:

Phenotype	Genotypes	
J	I-J-	J J,
JOc	iiJ-	J Ji
Oc	iijj	J j
"-"	I-jj	ii j or J

to which the following model is applicable



that is the I gene is a dominant inhibitor of the Oc synthetic pathway.

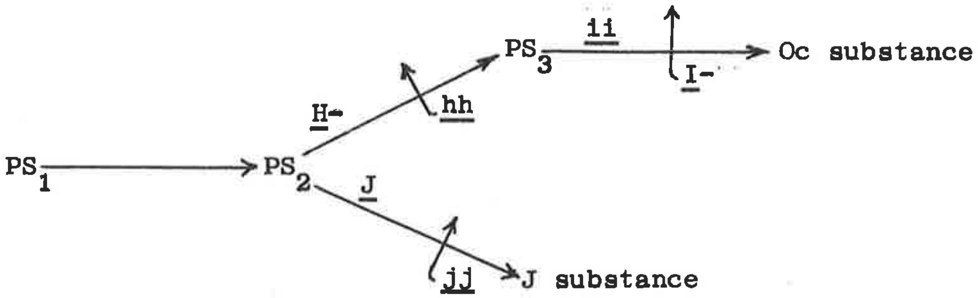
This model is compatible with Sprague's (1958a) data with the same exceptions as his multiple allele hypothesis; however it gives simpler genotype-phenotype relationships.

There are some observations not taken into account by the above model. Firstly, the associated occurrence of the J and Oc phenotypes in some populations of cattle. Thus Sprague (1958a) found that in two herds there was a significant positive association between the occurrence of the two substances (Table 23, p. 50) but this was not found in a third herd. Examination of cows from an abattoirs showed a significant negative association (this thesis, Table 23). Thus, on the basis of a two-locus model, the I and J loci would have to be closely linked.

Secondly, Sprague's (1958a) suggestion that his exceptions to his multiple allele hypothesis may be due to the segregation of alleles at a second locus could be incorporated into the model above (p. 75). However, in this case the locus is the third one involved. All three matings giving exceptions involved both parents *with* the Oc substance producing an offspring *without* it. Thus one could involve the alleles *H* and *h* at a third locus, where *H* has no effect on the synthesis of Oc substance but *hh* individuals synthesize none. The phenotype-genotype relationships proposed are:

Phenotype	Genotypes
J	I-J-H-, iiJ-hh
JOc	iiJ-H-
Oc	iijjH-
"-"	I-jjH-, iijjhh

and the following model is suggested:



This hypothesis could be tested since in those families where exceptions of the type discussed above are found, they are expected with a frequency of 0.25. Also, it may be possible to demonstrate two types of J and "-" individuals: those which have Oc substance in their secretions (semen, saliva) and those which do not.

These would presumably correspond with those that were H- and hh at the third locus. Due to the fact that no cattle anti-O reagent was available these tests were not carried out.

3. Transfer of soluble blood-group substances across the placental barrier

To explain the high proportion of mixed R/O phenotypes in a sample of sheep foetuses, it was proposed that there was transfer of soluble blood-group substances from the dam's circulation to the foetus (Appendix II). A large number of dam-foetus pairs were not available to test this hypothesis due to technical difficulties in obtaining such paired samples where the sheep were dressed. Since the cattle data does facilitate such a study, some information on placental transfer can be obtained. It was shown in Table 25 (p.52) that foetuses could be of different inhibition type from their dams and therefore it is apparent that the data are not consistent with a general placental transfer theory. Isolated cases due to disease cannot be discounted.

4. Transferrin mosaicism in twins

Datta and Stone (1963c) obtained evidence that four pairs of cattle twins out of 96 examined had transferrins that might represent mosaic patterns. For this to be so, the animals would have to be liver tissue chimeras, given that most serum proteins are synthesized in the liver, and their rarity compared with erythropoietic chimeras may be due to incompatibility between the transferrin-fomring tissues at the time when venous anastomosis occurs. Since it is extremely unlikely (P = 0.004) that the members of the six pairs of twins in Table 10 (p. 45) have the same transferrin type by chance alone, one may conclude that this is the result of venous anastomosis leading to transferrin mosaicism. Although these foetal twins are probably transferrin pattern mosaics, it is unlikely that they are liver tissue chimeras in view of the findings of Datta and Stone (1963c). Since differences in transferrin patterns are detectable in dizygotic twins 30 hours after birth, the transferrin mosaicism present at birth must be rapidly lost as a result of a turnover in the blood serum proteins.

5. The use of abattoirs material for blood group studies

The results of studies on abattoirs material presented in this thesis indicate that for blood groups and serum protein variants that do not exhibit dominance and which are expressed in foetuses, dam-foetus pairs represent an ideal source of incomplete family data, maternal foetal incompatibility data and population data. In addition to this, the foetal blood samples provide material for the study of the development of blood-groups and serum proteins.

Whilst abattoirs material can provide information on the above aspects of blood group and serum protein studies, the collection of this material has some disadvantages compared with the collection of farm material due to the extremely high noise level and adverse working conditions.

h. Phylogenetic relationships

Bison bison and *Bison bonasus*, the American and European bison have the same transferrin pattern which is similar to but not identical with TfA of *Bos taurus* and *Bos indicus* (Stormont, 1964; Braend and Gasparski, 1967). On the basis of blood-group studies by Stormont *et al.* (1961), Owen *et al.* (1958) and Miller and Stone (1955), and the observation that viable hybrids can be produced when *Bison bison* is crossed with domestic cattle, the F_1 females being fertile (males sterile) (Deakin *et al.*, 1942), it is likely that the bison should be in the genus *Bos*. Indeed, Roe (1951) classifies the American bison as *Bos americanus*.

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this

Banteng cattle are similarly cross fertile with domestic cattle and the F_1 females are fully fertile (males sterile) (Slipjer, 1951). Simpson (1945) classifies these as *Bibos banteng*, Walker (1964) regards them as *Bos banteng* and Zeuner (1963) gives them the status of subgenus i.e. *Bos* (*Bibos*) *banteng*. Morphologically, the animals resemble *Bos taurus* more closely than do *Bison bison* (Figure 18, p. 57). Observations on serum proteins and blood groups reported in this thesis (p. 57) indicate that banteng and domestic cattle are more closely related than their classification into separate genera would imply.

All 30 banteng sera examined showed a transferrin pattern that was indistinguishable from the domestic cattle type D_1. The serum of a single hybrid (Banteng male x Zebu female) animal was examined and this showed a transferrin pattern indistinguishable from AD_1. The male parent was D_1 and the female parent AD_2. Thus it seems likely that banteng cattle have an allele in the same series as that described in *B. taurus* and *B. indicus*. Whether there is any transferrin variation in banteng cattle in general cannot be determined from these 30 animals. Also, there is the possibility that the Australian population was derived from very few animals and other transferrin variants were absent from the foundation stock or were lost in subsequent generations. Both the Asiatic buffalo and banteng cattle of Australia were introduced from Timor into Melville Island off the northern coast of Australia in 1824-25 and were subsequently transferred to Port Essington on the mainland in 1838 (Letts, 1961). Although the numbers of these animals originally introduced is unknown, the number of banteng at least, was low. After revisiting the abandoned settlement in 1874, John Lewis reported in 1914 that:

> "We found quite a number of English cattle on the Peninsula [Cobourg Peninsula] but they did not thrive there very well. Also a number of Sourabaya cattle [banteng], Timor ponies and a large number of buffaloes. I estimated the number of buffaloes on the Peninsula and outside at about 20,000."

The 30 banteng sera all had an albumin zone not previously described in cattle and this has been called D. However, 11 animals also had an albumin zone that was electrophoretically indistinguishable from the A albumin zone of *Bos taurus* and *Bos indicus*. The hybrid animal had albumin type BD (Alb D x Alb B ⟶ Alb BD) and there was no evidence for the production of interaction products. Thus albumin A of cattle and banteng are probably determined by homologous genes.

The presence in banteng of a similar JOc inhibition system to that of cattle cannot be interpreted as indicating a close relationship between domestic cattle and banteng cattle since similar systems have been described in goats and the Norwegian elk. Whilst the banteng JOc system is superficially similar to the cattle JOc system, it shows a noticeable difference with respect to the presence of anti-sheep O in the serum. The regular association of anti-sheep O antibody with the absence of Oc substance has not been found in *Bos taurus* where the presence of the antibody is apparently determined by a dominant gene (Sprague, 1958b).

Haemoglobin was not available from the Australian banteng cattle; however, Vella (1958) has examined banteng and buffalo haemoglobin. Although these were not compared with domestic cattle haemoglobins, examination of his figure as well as a personal communication received from Dr. K. Bell who has also examined albumins and haemoglobins of banteng blood at the University of Queensland, indicate that banteng cattle have a haemoglobin with different electrophoretic mobility from the cattle variants (called B_{banteng}) but some individuals also have cattle haemoglobin B.

Dr. Bell has also examined the milk proteins of seven banteng cows. They all had an α-lactalbumin different in mobility from that of Bos taurus and which was designated C. No polymorphism was demonstrated. The β-lactoglobulins showed variation and three phenotypes designated E, EF and F have been found. These are different from the previously described variants A, B, C and D in domestic cattle. The α-casein showed variation between individuals, some having type C indistinguishable from cattle C and some having $C_{banteng}$, a slower variant. β-casein variation was also observed; a new variant named A_4 and type A_2 indistinguishable from cattle A_2. No polymorphism was demonstrated in K-casein; all were the same as cattle B. As with transferrin, the absence of variation in some of these milk proteins which commonly show variation in other cattle may be related to a small foundation population.

In conclusion, the evidence from studies on blood and milk proteins in banteng cattle support the contention that these animals are probably more correctly classified as Bos banteng.

The Asiatic water buffalo found in Australia is the same strain as the Philippine Kerbau (Letts, 1961). The transferrin variants found were not the same as any of the known cattle-variants and it is probable that the polymorphism is the same as that reported briefly by Makaveev (1966). The variants themselves will therefore, not be discussed. The interesting characteristic of the buffalo transferrin patterns is that they are much more clearly resolved into five zones than cattle D_1 and D_2 (Figure 20, p. 59) and in this respect are similar to the transferrin found in the European and American bison. Whether this five-zone system is a general characteristic of the Tribe Bovini is yet to be determined; however, present information indicates that it is. Studies on the transferrins of the Tibetan Yak and the Gaur and Gayal cattle of Southern Asia would be of particular interest in this respect.

SECTION B

THE ASSOCIATION OF SERUM TRANSFERRIN AND J BLOOD GROUP TYPE WITH PRODUCTION

I. LITERATURE REVIEW

a. Production characters and blood groups in animals

If the numerous known blood-group systems in animals represent selectively balanced polymorphisms and not transient polymorphisms then it may be possible to find blood-group genes which are associated with superiority in fitness or production capacity. Presumably natural selection may act on some pleiotropic effect of the blood-group genes, and one of the more obvious possibilities here is disease susceptibility and resistance. Thus, as early as 1921 Buchanan and Higley investigated the ABO blood groups in people suffering from a number of organic diseases. Although the findings of these workers were negative, subsequent studies by a number of workers revealed significant associations between disease susceptibility and the ABO blood-group system of man. Some of these are tabulated in Race and Sanger's book (1962). The significance of most of these associations in the maintenance of the blood group polymorphism must however be regarded as doubtful since the diseases do not manifest themselves before reproductive age is reached and even then are not generally debilitating. Although the human investigations are of inherent interest, studies on chickens have been more intensive and have revealed strong selective forces acting on blood-group genes . Gilmour (1954) and Briles et al. (1957) concluded that the maintenance of heterozygosity at the B blood group locus in highly inbred lines of chickens indicated that the B polymorphism was selectively balanced. Other blood group loci in inbred chickens also retain heterozygosity to a higher degree than might be expected (Gilmour, 1959) and B-locus heterozygote superiority in viability and reproduction during inbreeding has been demonstrated by Briles (1956), Gilmour (1960) and Briles and Allen (1961): homozygotes produced fewer eggs than heterozygotes in some lines of poultry but not others. The effect of the B blood group system was not confined to heterozygote superiority in inbred lines, since Allen and Gilmour (1962) found a significant difference in overall fitness between heterozygotes with a common allele in crossbred chickens. Robertson (1966) reported that a number of poultry breeding firms had closed down their blood grouping operation after several years work because of lack of results and it was considered that some of the significant associations observed between blood groups and economic characters in poultry may only apply to the particular population studied. However, the findings with chickens provide a stimulus for a search for similar associations in other animals.

A study of the relationship between serological and other traits in turkeys (Stevens, 1965) revealed one significant association in 354 separate comparisons involving ten alleles at three loci and seven production characters. Hybrid turkeys with the allele a^{A_1D} had significantly longer shanks than those without. In inbred lines there was evidence for a general selective advantage of A system heterozygosity and for an association of the Alb^A allele with large size in males and small size in females. Stevens (1965) concluded that the difference in results between the random-bred and selected lines and between domestic and hybrid turkeys demonstrated the dependence of blood group effects on the genetic background of the population.

Bender (1965) investigated blood groups in two species of trout. Results of studies on rainbow trout indicated a selective advantage for some blood group types within strains but a similar effect could not be found in brown trout.

Investigations with sheep failed to show any association of a number of production characters with the R-O and X-Z blood groups (Stansfield *et al.*, 1964). However, Evans and Turner (1965) claim to have detected a significant association of haemoglobin type with fertility, haemoglobin B animals producing more multiple births than those with haemoglobin A.

In cattle, the association between production or fitness characters with blood groups have been studied by numerous workers. The results of some of these studied were not available to the author but have been cited by Neimann-Sorensen and Robertson (1961) and Maijala (1966). These include Bouw (1958), Mitscherlich *et al.*

(1959), Larsen *et al.* (1959), Tolle (1959, 1960), Rendel (1959), Andresen *et al.* (1959), Smith and Pfau (1962), Munkacsi (1962) and Salerno (1963). A review by Neimann-Sorensen and Robertson (1961) however, indicates that: (1) Bouw (1958) did not demonstrate any association between the A, V or Z locus of a bull and its genetic value for fat percentage; (2) Mitscherlich *et al.* (1959) found that cows with blood-group factor M yielded less milk than those without it: similarly with O_3 and R. Y_2 and L were associated with a lower fat percentage and Q with a higher fat percentage; (3) the observations on M, Y_2 were confirmed by Tolle (1959); and (4) Rendel (1959) found statistically significant associations between some blood group genes and fat percentage, notably $\underline{B}^{BO_1Y_2D}$, an observation made also by Larsen *et al.* (1959) and Andresen *et al.* (1959).

Dunlop (1951) and Nair *et al.* (1955) investigated type defects such as bad rib, udder and teat conformation and their association with blood group antigens. No significant association was found. Laben and Stormont (1958) could find no evidence for the preferential survival of calves heterozygous at the B, F-V or Z blood group loci during inbreeding. However, Conneally (1962) and Conneally *et al.* (1961) found that during inbreeding there was an excess of Z/z calves from segregating matings at the Z locus and Fowler *et al.* (1963) examining A, F-V, L, S and Z blood groups for maternal-foetal incompatibility found that in segregating matings involving the F-V locus (F-V x FF) there was a significant excess of FF offspring.

The observation that the A antigen had low frequency in Friesian cattle and high frequency in Jersey cattle led McClure (1952) to investigate a possible association of the A antigen with fat percentage but none was found. However, Barr (1960) also investigated the A antigen and found the heterozygous animals gave 464.5 lb. more milk and 10.6 lb. more fat than homozygous animals. Investigations into production characters and blood group antigens controlled by genes at other loci have also revealed associations. Conneally (1962) found that the Z locus had a heterotic effect on milk production and $\underline{Z}^{z}$ had a significant additive effect on fat percentage. In a second herd examined none of these associations were observed and Conneally and Stone (1965) could not confirm the observation. Barr (1960) observed that calves with the Z antigen grew significantly faster than those without it.

Investigations have also indicated an effect of the L locus on production. Rendel (1959, 1961) found that the L antigen was associated with a lowered fat percentage (0.09%) and Barr (1960) showed that in his sample, animals lacking the L antigen were significantly larger at birth and grew faster than those with the antigen.

One of the most consistent associations between a blood group antigen and production characters to be found in the literature is that between the phenogroups BO_1Y_2D' and BO_1Y_1D' and an increased fat percentage. Rendel (1959, 1961) compared groups of paternal half sibs from sires carrying $\underline{B}^{BO_1Y_2D'}$ and found a significant difference of 0.1 in the fat percentage of the groups. It was then predicted that differences of the order of 0.2% might be found between cows with and without the phenogroup since most of the sires used were heterozygous. Neimann-Sorensen and Robertson (1961) using a within half-sib progeny group analysis found that cows of the RDM breed with the phenogroup BO_1Y_1D' had a fat percentage 0.064 ± 0.015 higher than their contemporaries. Since this was associated with a negative effect on milk yield, a gain in fat yield could not be expected to result from selection of animals with this phenogroup. Subsequent studies by Conneally (1962) and Conneally and Stone (1965) also using a within progeny group analysis indicated that the BO_1Y_2D' phenogroup was associated with an 0.33 increase in fat percentage. A similar effect is reported to have been shown by Andresen <u>et al</u>. (1959) although to what extent this represents a repetition of the reports of Larsen <u>et al</u>. (1959) and Neimann-Sorensen and Robertson (1961) is not known to the author. Conneally (1962b) working with a different cattle population, and other workers (Smith and Pfau, 1962; Maijala, 1966) have been unable to confirm this finding.

It is apparent from the above discussion and from Table 30 that at least a dozen associations have been found by about six independent workers. Most have, however, been shown not to exist in

TABLE 30

A LIST OF BLOOD GROUP FACTORS OR ALLELES STUDIED FOR ASSOCIATION WITH PRODUCTION AND FITNESS CHARACTERS IN DAIRY CATTLE

Locus	Blood Group factor or Allele	Association Found With				Association Not Found With*			
		Fat %	Milk Yield	Fat Yield	Other	Fat %	Milk Yield	Fat Yield	Other
A	A		10	10		2,1,3,12,10	2,1,3	3	9,10
J	J^{cs}	7,11			4	1,3,10	1,3,10	3,10	10
L	L	5,7,11			10	2,1,3	2,1,3	3	9
M	M		5,6			1	1		
F-V	F				9	2,3,10	2,3,10	3,10	13,10,9
	V					2,1,3,12	2,1,3	3	
Z	Z	3			3,8,10	2,1,3,12	2,1,3	3	13,9,3
	z								
SV	S1(S)	4				1,3,10	1,3,10	3,10	10,9
	S2(H')					2,1	2,1		
	V'(U2)					1,3	1,3	3	
C	C1					1	1		
	C2					1	1		
	E(D1)					1	1		
	R		5			1	1		
	W					1	1		
	X1					1	1		
	X2					1	1		
B	B					1,3	1,3	3	13
	b								
	O1					2	2		
	Y2	5,6				1,3	1,3	3	
	E_1'					1	1		
	E_3'					2,1	2,1		
	I'					1	1		
	IE_1'					1	1		
	GD'					1	1		
	BI'					1	1		
	PI'					2,1	2,1		
	O_1A'	2					2		
	BO_1					1	1		
	GY_2E_1'					1,3	1,3	3	
	BO_1I'								
	BOI'A'					1	1		
	BO_1Y_2D'	3,7,11				2,3,4	2,3	3	
	BO_1Y_1D'	1							
	$Y_2D'E_1'$					1	1		
	$Y_1D'E_3'$					1	1		
	QOJ'K'					1	1		
	$QD'E_1'$					1	1		
	$O_1TE_3'K_1'$	1							
	$BOY_1'A'E_3'$					1	1		
	$BGKOY_2A'$					1	1		
	$IOQA'E_1'K'$					1	1		
	BOY2A'E3'					1	1		
	BGKOY1A'E3'K'					1	1		
	O3	5							
	Q	5							

Source List

1 Neimann-Srensen and Robertson (1961), Larsen et al (1959)
2 Maijala (1966)
3 Conneally (1962), Conneally and Stone (1965)
4 Smith and Pfau (1962)
5 Mitscherlich et al (1959)
6 Tolle (1959)
7 Rendel (1959)
8 Conneally et al (1961)
9 Fowler et al (1963)
10 Barr (1960)
11 Rendel (1961)
12 Bouw (1958)
13 Laben and Stormont (1958)

* Some negative results are not presented since specific blood groups were not mentioned.

Smith and Pfau (1962)

one or more other populations studied supporting the view of Stevens (1965) that the effect of blood-group loci on other heritable traits in animals is dependent upon the genetic and physical environment of the animals. Table 30 lists the blood-group loci and alleles studied for associations with production and fitness characters on dairy cattle by various workers and their results. From the table it can be seen that where a blood group has been studied by more than one laboratory or in different populations by the same laboratory any apparent association has as many or more negative reports. The failure to find an association in a particular population may not be very meaningful as far as the real effect of the blood group is concerned. Thus, even $\underline{B}^{BO_1Y_2D'}$ has as many negative observations as positive. If one disregards the negative observations then the BO_1Y_2D', Y_2, L, M and J blood groups are those where more than a single observation has been made on the same association, viz. BO_1Y_2D' and J^{CS} with increased fat percentage, Y_2 and L with a lowered fat percentage and M with a lowered milk yield.

A consideration of the recorded effects of the J locus is more relevant to the work reported in this study. In Table 38 the allele at the J locus has been recorded as J^{CS} since the blood groups were obtained using an anti-J reagent on red cells. These will actually include those classified as J^{CS} and those classified as $J^{CS}Oc$ using two reagents (bovine anti-J and bovine anti-sheep O). Thus, Rendel (1959) and Rendel (1961) on two independent sets of data showed that animals with the J antigen on their red cells had a fat percentage about 0.04 higher than those without. Smith and Pfau (1962) found that animals of type J^{CS} had a withers height 1.4 cm higher than those of type J^{a}.

b. Production characters and transferrin type in dairy cattle

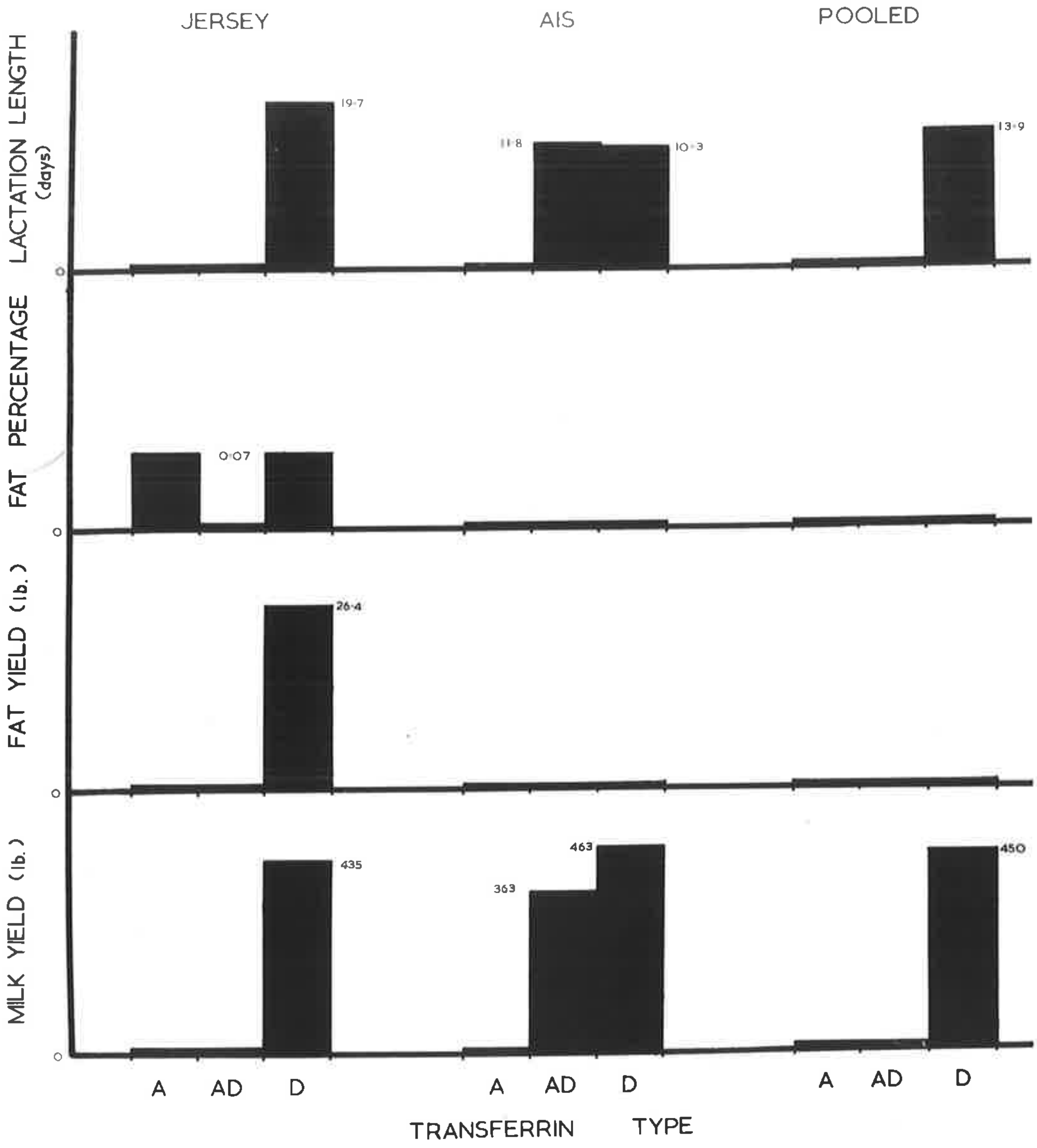
Although the evidence for an effect of transferrin type on fertility and fitness in cattle is not consistent, if some of the observed effects are real it is not unreasonable to expect an effect of the transferrin locus on production characters since dairy cattle production performance is closely related to their reproductive performance.

Ashton (1960b) examined the association of the transferrin type of bulls used in artificial breeding with their genetic value for milk yield as assessed by a contemporary comparison estimate (see Johansson, 1960; Searle, 1964; for review of sire proving techniques). Examination of 130 bulls used for artificial breeding composed of five breeds showed firstly that as a group they had significantly higher contemporary comparison values than 1028 bulls located on dairy farms (25.0 gallons versus 10.3 gallons of milk). Within the artificial breeding group, 39 transferrin A, 61 AD and 30 D animals had mean contemporary comparisons of 12.2, 26.8 and 38.2 gallons respectively, the mean value for A being significantly different from that for D. When the 130 bulls were broken down into their five breed categories a similar trend in mean production was observed in all cases. Assuming a gene frequency of 0.5 for $\underline{Tf}^A$ and $\underline{Tf}^D$ it was calculated that a sample of transferrin A cows from an A bull might be expected to have a mean milk yield 52 gallons lower than a sample of D cows from a D bull. Finally it was estimated that the transferrin locus might be responsible for at least 17 per cent of the total genetic variation in milk yield.

Ashton *et al.* (1963, 1964) further investigated the effect of the transferrin locus on production by examining 225 Jersey cows in 13 herds in the Nambour region of Queensland and 436 Australian Illawarra Shorthorn (AIS) cows in 24 herds in the Kingaroy region of Queensland. The transferrin type/production data were analysed statistically using a multiple linear regression analysis where the independent variables were herd and transferrin type and the dependent variable was milk yield, fat yield, fat percentage and lactation length in four regression analyses. The findings for each breed and for the pooled data are set out in Figure 21. The effects are similar for each breed except that there is evidence that the effect of D is recessive to A in the case of Jerseys and dominant in the case of AIS. It was shown that the primary effect of the transferrin locus in increasing milk yield was not on lactation length alone since the increase in lactation length could not account for the whole increase in milk yield. Thus, the effect of the transferrin locus on milk yield was considered to be the result of both an increase in lactation length and an increase in

FIGURE 21.

A graph of the results of Ashton *et al*. (1963, 1964). Only significant differences are shown and all results are expressed as the difference between transferrin types D, AD and A, i.e. D-A and AD-A.


JERSEY
AIS
POOLED
LACTATION LENGTH (days)
19·7
11·8
10·3
13·9
FAT PERCENTAGE
0·07
FAT YIELD (lb.)
26·4
MILK YIELD (lb.)
435
363
463
450
0
0
0
0
A
AD
D
A
AD
D
A
AD
D
TRANSFERRIN TYPE

average daily yield. The proportion of the genetic variation of milk yield attributable to the transferrin locus was calculated as 10.4 per cent for Jerseys and 6.0% for AIS and it was estimated by these workers that if homozygosity for $\underline{Tf}^{D}$ was obtained, the milk yield could be increased by about five percent. Actually, if one calculates the proportion of genetic variance attributable to the transferrin locus by the method described by Falconer (1960) the figures obtained are 8.4 per cent for Jerseys and 5.1 per cent for AIS and the expected increase in milk yield resulting from homozygosity at the transferrin locus is 8.7 per cent and 2.4 per cent for Jerseys and AIS respectively.

Ogden (1961) cited unpublished results which are similar to the observations of Ashton _et al_. (1963, 1964). For the Ayrshire breed, it was estimated that about four per cent of the total genetic variation in milk yield was influenced by the transferrin locus. Similar findings have been published by Osterhoff and van Heerden (1964b). Rausch (1963) and Rausch _et al_. (1963) also reported a significant association of transferrin type with milk production; however, this was not the same association as that described above. In 215 cows there was a significant negative correlation between the presence of the $\underline{Tf}^{E}$ allele and milk production. That is animals without $\underline{Tf}^{E}$ produced significantly higher milk yields than those with $\underline{Tf}^{E}$.

More recently, other workers were not able to find either of the above associations (Datta and Stone, 1963a; Datta *et al*., 1965).

From the above considerations it must be concluded that there is as much evidence for an effect of the transferrin locus on milk yield as there is for other loci on fat percentage. However, the lower heritability of milk yield makes it unlikely that many workers will be able to find similar effects in their particular samples. Also, if the real effect of the $\underline{Tf}^{D}$ allele on production can be attributed to either $\underline{Tf}^{D_1}$ or $\underline{Tf}^{D_2}$, then differences in the frequency of these alleles in different samples will greatly influence the results. However, given the same gene frequency structure it should be possible to repeat the results of other workers if the genetic backgrounds of the populations are similar.

Jamieson + Robertson
1967

II. METHODS

a. The dairy cattle population

In South Australia, dairy cattle may be divided into purebred and grade animals according to whether or not they have a pedigree registered in the books of the appropriate society. Within the purebred population, a number of different breeds exist; however, the majority of animals are restricted to the major breeds, viz. Jersey, Friesian, Guernsey and Australian Illawarra Shorthorn in that order. The herd is the most conveniently defined subpopulation, consisting of a finite number of animals under single management; however, other subpopulations can be defined such as half-sib families and full sib families, the members of which may or may not be in the same herd.

The dairy cattle population studied in this thesis is defined as all the Jersey and Friesian purebred cows in South Australia which are in herds involved in the monthly milk-testing scheme of a Herd Improvement Association and which belong to half sib families of ten or more individuals all lactating at the same time in the same herd. All cows in this population were bled and the number of animals involved, and the distribution of the half-sib families in the herds are given in Table 31.

Two successive years' production figures were analysed. The 1963 results included all cows calving between January 1963 and

TABLE 31

THE NUMBER OF ANIMALS IN THE HERDS AND PROGENY GROUPS OF THE JERSEY AND FRIESIAN POPULATIONS IN 1963 AND 1964

Jersey

Herd No.	No. in herd 1964	No. Cows bled	Prog. Group No.	Number in group		1964	c.f.	1963
				1963	1964	$ New	Omitted	Net
1	20	13	1	13	13	0	0	0
2	59	32	2	29	25	3	7	- 4
3	44	30	3	19	20	2*	1	1
			4	8	8	1	1	0
4	29	25	5	16	17	6	5	1
5	60	13	6	5	12	7	0	7
6	41	32	7	7	8	3	2	1
			8	11	9	0	2	- 2
			9	11	11	0	0	0
7	52	26	10	5	4	4	5	- 1
			11	15	13	2	4	- 2
8	36	26	12	12	12	0	0	0
			13	12	14	2	0	2
9	34	22	14	12	13	1	0	1
			15	9	9	0	0	0
10	38	10	16	10	9	0	1	- 1
11	24	10	17	10	6	0	4	- 4
12	61	30	18	13	13	3	3	0
			19	4	12	8	0	8
13	68	45	20	12	12	0	0	0
			35	21	31	10	0	10
14	31	31	21	14	16	4	2	2
			22	12	9	1	4	- 3
15	45	12	23	11	12	1	0	1
16	58	33	24	19	31	13	1	12
17	54	22	25	10	10	2	2	0
			26	10	7	0	3	- 3
18	41	11	27	5	11	6	0	6
19	20	18	28	14	18	4	0	4
20	47	22	29	11	10	0	1	- 1
			30	7	8	4	3	1
21	48	31	31	23	31	8	0	8
22	12	12	32	7	11	5	1	4
23	46	30	33	13	17	4	0	4
			34	10	12	3	1	2
24	35	13	36	-	12	12	0	12
Total	1003	549		420	486	119	53	66
Mean	41.8	22.9		12.0	13.5			

Friesian

Herd No.	No. in herd 1964	No. Cows bled	Prog. Group No.	Number in group		1964	c.f.	1963
				1963	1964	$ New	Omitted	Net
1	27	19	1	9	9	0	0	0
			2	10	7	0	3	- 3
2	28	20	3	14	19	5	0	5
3	30	12	4	6	11	5**	0	5
4	63	44	5	21	19	0	2	- 2
			6	20	17	2	5	- 3
5	58	60	7	8	8	0	0	0
			8	8	9	3	2	1
			9	33	28	1	6	- 5
6	52	44	10	12	12	1	1	0
			11	13	14	1	0	1
			15	-	14	14	0	14
7	60	51	12	22	10	0	12	-12
			13	13	19	9	3	6
8	27	22	14	18	21	4	1	3
Total	345	272		207	217	45	35	10
Mean	43.1	34.0		14.8	14.5			

$ All animals entering the second analysis were 2 years old except:

* Both mature cows, 5 and 7 years old.

** All 3 years old.

December 1963 inclusive, and the 1964 analysis included all cows calving in the same period in 1964. Although 821 blood samples were taken, wastage occurred because some animals were sold or died before their subsequent performance data were recorded. Some animals qualified for inclusion in both analyses, some in the first only, and some in the second only: the relationship between cows in the first and second analyses is given in Table 31.

b. Dairy cattle performance parameters

Production performance data were extracted from the Official Herd Testing records compiled annually by the Dairy Cattle Improvement Association administered by the Department of Agriculture. For each animal the following data were recorded:-

1. HERD LEVEL, defined as the average yield of fat per cow per lactation in a given herd in the year of the survey, was taken directly from the published herd testing report. Every animal in a given herd was allocated the same herd level value.

2. PROGENY GROUP LEVEL, in a manner analogous to herd level, referred to the average yield of fat per cow per lactation in a given group of half sibs. Every animal in the same progeny group therefore, had the same progeny group value.

Both the herd and progeny group classifications were based on a mean fat yield in preference to milk yield or fat % because this is the basis of commercial classification.

3. AGE, as recorded by the Department of Agriculture had a class interval of 6 months from 2 to 5 years and 12 months from 5 years onwards. The age classification was made on the first day of lactation.

4. LACTATION LENGTH is recorded by the Department of Agriculture in days commencing five days after calving. However, since the measurement has a class interval of 30 days it was recorded as 1, 2, or up to 10 30-day periods for this analysis.

5. MILK YIELD measured as the total weight of milk produced by a cow in the lactation under consideration, was based on a monthly weighing of milk yielded by a cow in one evening and the following morning. Night and morning milk weight was read to the nearest tenth of a pound and their combined weight taken to the nearest pound. The total weight of milk yielded in a month was estimated by multiplying the observed production over one day by the number of days of production in that month.

6. FAT YIELD was an estimate of the total weight of fat produced in a lactation based on estimates of fat yield for each month given the monthly estimates of milk yield and fat percentage.

7. FAT PERCENTAGE for a given lactation was derived from estimates of the total milk and fat yields for the lactation commencing in the year of the survey. The monthly fat test was carried out using the Australian Standard Method number N26 (1958).

8. CALVING INTERVAL was the interval in months between the calving resulting in a given lactation and that resulting in the previous one.

9. MONTH OF CALVING probably influences subsequent production (South Australian Annual Herd Testing Reports). For the purposes of this analysis an integer from 1-12 was allocated to an animal according to the month of year in which calving occurred. That is, January = 1.0 and December = 12.0.

10. NUMBER IN HERD was the number of cows in a given herd in the year of survey.

c. Analysis of data

Analysis of the relationship between both transferrin type and J blood group type and production performance measured in several ways was carried out with the aid of the Control Data Computers 3200 (C.S.I.R.O., Adelaide), 3600 (C.S.I.R.O., Canberra) and 6400 (University of Adelaide). Subroutine "matinv", used for matrix inversion in the multiple linear regression analysis, was obtained from the C.S.I.R.O. program library.

The different methods of statistical analysis used below were not selected to extract different information from the data in each case. Most were selected because they have been used at some time by other workers to examine data for associations between blood groups and production parameters. The results of the different analyses can therefore be compared with those obtained by other workers and can also be compared with each other.

1. Comparison of the mean performance of the three transferrin types

As a first approach, variance ratios were obtained by comparing between transferrin type mean squares with within transferrin type mean squares for seven parameters of dairy cattle performance, namely: fat %, fat yield, milk yield, lactation length, calving interval, month of calving and herd level. This analysis is therefore similar to that carried out by Datta _et al_. (1965) and by White and Banfield (1967). In an attempt to make the comparisons under more uniform conditions further analyses were made in which restrictions were introduced:

(i) The above analysis was carried out within two age groups; first lactation (2 years old) and mature cows (5 years and over). Other age classifications (3 years and 4 years) were not included due to the low numbers of animals in the subclasses.

(ii) Taking into account the progeny group structure of the population, the data were analysed in two ways:

(a) As a two way classification into progeny groups and transferrin types

For this analysis, N cows were classified into their half-sib families, and into three groups according to their transferrin type. The number of animals within a particular progeny group and transferrin class (subclass) varied from 0-23;

however, the analysis was carried out assuming equal numbers in subclasses although there were neither equal nor proportionate numbers in subclasses ($P < 0.001$ for homogeneity χ^2 in each case). This point is discussed on page 110.

Variance was partitioned as follows:

Variance due to:	Degrees of freedom	Expected mean square
Between sires	n-1	$\sigma_w^2 + 3\sigma_S^2$
Between transferrin types	2	$\sigma_w^2 + n\sigma_T^2$
Sires x transferrin type	2(n-1)	$\sigma_w^2 + \sigma_{ST}^2$
Within subclasses	N-3n	σ_w^2
Total	N-1	

where: σ_{ST}^2 = component of variance due to the interaction between progeny group and transferrin type

σ_S^2 = between sires component of variance

σ_T^2 = between transferrin types component of variance

n = number of sires

3 = number of transferrin types

The variance ratios were all determined as the ratio of the relevant mean square to the within subclass mean square.

For this analysis only those characters which represented individual production characters could be used, viz. fat %, fat yield, milk yield, lactation length, calving interval and month of calving.

(b) Using the same six performance parameters listed for the analysis of variance above (a), the mean difference between transferrin classifications, its accompanying standard error and the homogeneity of the differences in the separate progeny groups were estimated using the method described by Neimann-Sorensen and Robertson (1961). This method is based on a hierarchical classification of cows into half-sib families and transferrin types and estimates are made of the difference in production between transferrin types within the progeny groups.

An estimate was made of the following differences between transferrin types: $D_1 = AD - A$, $D_2 = D - A$, $D_3 = D - AD$, $D_4 = D - (AD + A)$, $D_5 = (D + AD) - A$.

2. Studies of covariation

A large number of simple linear correlation coefficients were determined for pairs of variates.

In the multiple linear regression analysis the traits fat %, fat yield, milk yield and lactation length were considered dependent variables and transferrin type, age, calving interval, month of calving, herd level, progeny group level and number in herd as independent variables. To aid comparison, this classification was based on that of Ashton, Fallon and Sutherland (1964), so that the same dependent variables were used although they used only two independent variables, namely transferrin type and herd.

When multiple significance tests (t-tests) were carried out, the significance levels for the whole set corresponded to single test levels of α given by $(1 - \alpha)^n = 0.95$ (0.99, 0.999), (Sorensen and Robertson, 1961) where n was the number of simultaneous tests made on a set of data.

3. Simulation studies

If the association between transferrin type and performance is spurious due to between progeny group differences (see discussion p.112), then random assignment of transferrin genotypes to individuals within progeny groups, given the progeny group genotypic frequencies, should not break this association.

To test this hypothesis such a procedure was carried on the transferrin data examined in this thesis. The technique used may be described briefly as follows; the computer is given the genotype frequencies for each progeny group and the transferrin types are allocated at random within these as follows:

$$f_1 = f_A$$

$$f_2 = f_A + f_{AD}$$

$$f_3 = f_A + f_{AD} + f_D = 1$$

As each animal's production data is read, the machine generates a random number between 0 and 1 and compares this with f_1. If the random number is less than or equal to f_1 the animal is assigned transferrin type A. If the random number is greater than f_1 it is compared with f_2. If it is less than or equal to f_2 the animal is assigned transferrin type AD. If it is greater than f_2 the animal is assigned transferrin type D. In this way genotypic frequencies closely agreeing with those supplied as data are obtained. Table 32 shows an example of the mean numbers allocated to subclasses in three simulations.

TABLE 32

MEAN NUMBERS ALLOCATED TO SUBCLASSES IN SIMULATION EXPERIMENTS, 1964 FRIESIAN DATA

Progeny Group	Tf A		Tf AD		Tf D		Total Actual and Simulated*
	Actual	Simulated⌿	Actual	Simulated⌿	Actual	Simulated⌿	
1	2	3	6	4	1	2	9
2	1	2	5	5	1	1	7
3	4	4	10	9	5	6	19
4	1	3	4	2	6	6	11
5	6	5	9	10	4	4	19
6	1	1	12	13	4	3	17
7	0	0	2	1	6	7	8
8	2	2	4	3	3	3	9
9	1	1	20	20	7	7	28
10	2	2	8	8	2	2	12
11	11	11	3	3	0	0	14
12	7	5	3	5	0	0	10
13	10	10	7	8	2	1	19
14	0	0	9	6	12	15	21
15	8	9	6	5	0	0	14
Total	56	58	108	102	53	57	217

⌿ This is the mean of 3 simulations taken to the nearest whole no.

* The total in simulation experiments always equals the actual total.

III. RESULTS

a. Performance data analysed with respect to transferrin

1. Comparison of means

(i) Analyses of variance

The results of the analyses of variance are given in Table 33. For all performance parameters used in two years and both breeds, the major source of variation was between progeny groups. Since the progeny groups are distributed among a number of herds (Table 31, p. 95), it is probable that the between progeny group variance has a large component contributed by the between herd differences. The progeny group means are given in Appendix III (Tables 61-64, p.148).

Both analyses of variance reveal significant variation of fat percentage, milk yield and fat yield between transferrin types; however in the analysis in which the error variation is partitioned from that between progeny groups, this significant variation is revealed at a lower level of probability.

The analyses of variance where animals were classified into various age groups are given in Appendix III (Tables 47-60, p.148) together with the results of the examination of the differences between the mean production of transferrin types; however Table 34 summarizes these results. Significant differences were found between the means of transferrin types in

TABLE 33

COMPARISON OF THE MEAN PRODUCTION OF TRANSFERRIN TYPES BY ANALYSIS OF VARIANCE

Production Trait	Year	Jersey				Friesian			
		Analysis $ without restriction	Two-way classification into progeny Groups and Transferrin Types			Analysis $ without restriction	Two-way Classification into Progeny Groups and Transferrin Types		
		$1963-F^{2}_{417}$ $1964-F^{2}_{483}$	Between Transferrins $1963-F^{2}_{315}$ $1964-F^{2}_{378}$	Between Sires $1963-F^{34}_{315}$ $1964-F^{35}_{378}$	Sires by Transferrins $1963-F^{68}_{315}$ $1964-F^{70}_{378}$	$1963-F^{2}_{204}$ $1964-F^{2}_{214}$	Between Transferrins $1963-F^{2}_{165}$ $1964-F^{2}_{172}$	Between Sires $1963-F^{13}_{165}$ $1964-F^{14}_{172}$	Sires by Transferrins $1963-F^{26}_{165}$ $1964-F^{28}_{172}$
Fat %	1963	3.78*	6.72**	10.98***	0.79	5.23**	8.22***	8.52***	1.71
	1964	5.37**	7.67***	7.71***	0.61	1.82	3.16	10.80***	1.72
Fat Yield	1963	1.68	2.88	9.60***	2.88	0.66	1.06	9.73***	1.31
	1964	1.71	2.41	7.38***	0.63	2.57	4.12*	10.35***	0.92
Milk Yield	1963	1.33	1.95	6.90***	1.95	1.56	2.47	10.69***	0.75
	1964	0.62	0.79	5.61***	0.65	5.82**	9.41***	11.02***	0.71
Lactation Length	1963	0.53	0.51	1.44*	0.51	0.93	1.29	0.87	0.30
	1964	0.86	0.86	1.88**	0.51	0.46	0.50	2.82**	0.71
Calving Interval	1963	1.99	2.48	4.76***	2.48	0.93	1.52	10.26***	1.33
	1964	1.35	1.66	4.93***	0.61	0.24	0.35	9.23***	0.47
Month Calving	1963	1.14	1.21	2.19***	1.21	0.29	0.30	1.70	0.83
	1964	1.89	2.03	2.67***	0.67	0.12	0.74	1.57	1.43
Herd	1963	2.50	-	-	-	10.63*	-	-	-
	1964	4.94*	-	-	-	13.56**	-	-	-

$ For the production parameter 'herd level', the following degrees of freedom apply:

Jersey	$1963-F^{2}_{20}$	Friesian	$1963-F^{2}_{5}$
	$1964-F^{2}_{21}$		$1964-F^{2}_{5}$

* $.01 < P < .05$

** $.001 < P < .01$

*** $P < .001$

TABLE 34

SIGNIFICANCE LEVELS OF THE DIFFERENCES BETWEEN THE MEAN PRODUCTION OF TRANSFERRIN TYPES $ ≁

Production Trait	Year	Jersey										Friesian									
		Total Population		Subdivision of population according to age								Total Population		Subdivision of population according to age							
				Subdivision 1.				Subdivision 2.						Subdivision 1.				Subdivision 2.			
				First Lact.		> 2 Years		Mature Cows		< 5 Years				First Lact.		> 2 Years		Mature Cows		< 5 Years	
		AD-A	D-A	AD-A	D-A	AD-A	D-A	AD-A	D-A	AD-A	D-A	AD-A	D-A	AD-A	D-A	AD-A	D-A	AD-A	D-A	AD-A	D-A
Herd Level	1963									**		**	***			**	**	*	**		
	1964	**	*							**		***	***	*	***	**	**			*	**
Fat %	1963		**				*					* –	***–			* –	** –				**–
	1964	*	**			*	**		**							* –	* –				
Milk Yield	1963																				
	1964											***	*			**	*	**			
Month Calving	1963							** –										**	**		
	1964																				

$ Extracted from tables 47-60 in Appendix III.

≁ There were no significant differences between TfD and TfAD means for any of the production traits measured and there were no significant differences between any transferrin types for fat yield, lactation length, or calving interval.

The direction of the difference is positive unless otherwise indicated by –

*	$.01 < P < .05$
**	$.001 < P < .01$
***	$P < .001$

both the Jersey and Friesian populations for the production parameters herd level, fat percentage, milk yield and month-of-calving as follows:

(a) In both breeds, herd level among cows of transferrin type AD and D was significantly higher than those of type A.

(b) The differences between the mean fat percentage of the transferrin types are in opposite directions for each breed. That is, transferrin A cows produced milk with a significantly higher fat percentage than transferrin D cows in the Friesian breed but the relationship was reversed in Jersey cows.

(c) Transferrin D and AD Friesian cows produced significantly more milk than transferrin A cows in 1964.

(d) In both the Jersey and Friesian mature cows in 1963, transferrin types had significantly different mean month-of-calving values. Mature Jersey cows of transferrin type AD had a significantly lower month-of-calving mean than those of type A. Mature Friesian cows showed the reverse relationship, that is transferrin D and AD animals had a significantly higher mean month-of-calving than those of type A.

With the exception of the month-of-calving effects, all significant differences were detectable in the overall population as well as in one or more of the age group subpopulations. The significant differences were not consistently found in any single age group although they were rarer in the first lactation age group. This is surprising since the heritability of production traits have been generally found to be higher in this age group and also the first lactation animals represent an unselected population as far as production traits are concerned. It is possible that there is an interaction between transferrin type and the environment (management) of the different age groups.

There were no significant differences between transferrin types AD and D for any of the production traits measured and there were no significant differences between any of the transferrin types for the parameters fat yield, lactation length or calving interval in either breed in both years. Further, in the Jersey sample there were no significant differences between the mean production of any transferrin types in animals in their first lactation.

(ii) Neimann-Sorensen and Robertson analysis

There were no significant production differences between transferrin types in the 1963 or 1964 analysis for Jersey cows (Table 35) and D_1 and D_5 are the only differences which show significant heterogeneity between progeny groups for any performance parameters.

The analysis of the 1963 Friesian data showed a significant difference between the fat percentage of milk produced by transferrin D and transferrin A cows, however the difference was

TABLE 35

COMBINED ESTIMATES OF THE DIFFERENCE BETWEEN TRANSFERRIN GENOTYPES WITHIN PROGENY GROUPS, WITH TESTS FOR THE HOMOGENEITY OF THE DIFFERENCES IN THE GROUPS (METHOD OF NEIMANN-SORENSEN AND ROBERTSON, 1961)

(a) Jersey

Difference between transferrin types	Year	Fat %			Fat Yield			Milk Yield			Lactational Length			Calving Interval			Month Calving			Degrees of freedom for homogeneity tests
		Mean	S.E.	V.R.≁	Mean	S.E.	V.R.≁	Mean	S.E.	V.R.≁	Mean	S.E.	V.R.≁	Mean	S.E.	V.R.≁	Mean	S.E.	V.R.≁	
D_1=AD-A	1963	0.037	0.046	1.42*	-17.04	8.90	1.58*	-40.01	16.98	1.21*	-0.140	0.123	0.90	0.035	0.622	1.08	-0.320	0.325	1.23*	F^{34}_{315}
	1964	0.056	0.045	1.16*	- 6.47	12.02	0.71	-22.75	18.19	1.07	0.011	0.135	1.03	0.123	0.516	0.86	0.003	0.293	0.96	F^{35}_{376}
D_2=D-A	1963	0.205	0.179	0.06	17.91	16.29	0.50	4.98	31.46	0.47	-0.067	0.390	0.06	2.177	1.006	0.33	0.144	0.494	0.89	F^{34}_{180}
	1964	0.129	0.068	0.58	9.51	22.40	0.15	0.66	31.61	0.26	0.213	0.296	0.08	-0.062	0.872	0.57	0.518	0.490	0.45	F^{35}_{212}
D_3=D-AD	1963	0.103	0.145	0.18	24.47	15.59	0.56	31.41	29.66	0.59	0.051	0.348	0.14	1.138	0.900	0.47	0.587	0.491	0.53	F^{34}_{240}
	1964	0.062	0.062	0.69	16.19	15.87	0.33	25.37	29.62	0.24	0.124	0.245	0.14	0.605	0.798	0.42	0.831	0.436	0.45	F^{35}_{276}
D_4=D-(AD&A)	1963	0.135	0.111	0.18	17.78	13.13	0.63	13.46	24.85	0.65	-0.024	0.259	0.16	1.358	0.813	0.34	0.424	0.429	0.56	F^{34}_{385}
	1964	0.092	0.059	0.58	12.85	13.87	0.34	13.38	25.77	0.27	0.127	0.211	0.08	0.345	0.708	0.53	0.738	0.391	0.51	F^{35}_{450}
D_5=(D&AD)-A	1963	0.072	0.044	1.08	- 9.47	8.62	1.47*	-29.98	16.26	1.11*	-0.113	0.117	0.73	0.450	0.585	0.94	-0.204	0.313	1.15*	F^{34}_{385}
	1964	0.078	0.043	1.05	- 4.19	10.92	0.74	-20.40	16.68	1.12	0.043	0.124	0.96	0.200	0.488	0.95	0.103	0.281	0.96	F^{35}_{450}

(b) Friesian

D_1=AD-A	1963	-0.022	0.065	2.24**	-14.51	16.74	0.85	-20.67	43.77	0.56	-0.101	0.071	0.44	1.150	0.932	0.82	0.526	0.623	0.61	F^{13}_{145}
	1964	-0.184*	0.069	1.78*	-21.58	16.79	1.14	8.21	42.29	1.74	-0.182	0.161	0.61	-1.356	0.816	0.34	0.208	0.674	1.46	F^{14}_{149}
D_2=D-A	1963	-0.359**	0.090	2.18*	-61.03	23.38	0.87	-48.41	68.72	0.73	0.033	0.314	0.01	-0.109	1.398	1.22	0.114	0.927	1.21	F^{13}_{87}
	1964	-0.367**	0.101	1.90*	-41.89	26.57	0.33	- 0.92	77.20	0.22	0.121	0.434	0.14	-1.558	1.185	0.26	0.071	0.981	1.17	F^{14}_{94}
D_3=D-AD	1963	-0.124	0.063	2.23**	-33.27	17.04	1.80*	-45.35	48.79	1.10	0.081	0.178	0.03	-1.056	0.915	2.28**	-0.207	0.585	0.87	F^{13}_{87}
	1964	-0.077	0.063	1.56	-12.81	17.81	1.51	-21.52	49.93	1.26	0.048	0.256	0.65	-0.379	0.773	0.52	-0.042	0.570	1.53	F^{14}_{146}
D_4=D-(AD&A)	1963	-0.146	0.066	2.01*	-35.65	16.33	1.79*	-45.58	44.78	1.20	0.065	0.148	0.02	-0.859	0.912	2.16*	-0.075	0.566	1.12	F^{13}_{193}
	1964	-0.115	0.066	1.60	-21.33	16.68	1.47	-30.65	45.94	1.19	0.023	0.219	0.83	-0.742	0.744	0.51	0.019	0.582	1.36	F^{14}_{202}
D_5=(D&AD)-A	1963	-0.144	0.064	2.07*	-22.03	16.75	0.73	-21.64	42.10	0.53	-0.075	0.062	0.44	0.926	0.939	0.77	0.440	0.588	0.86	F^{13}_{193}
	1964	-0.217**	0.068	1.88*	-20.93	18.01	0.85	17.90	44.99	1.20	-0.110	0.196	0.41	-1.348	0.788	0.36	0.106	0.643	1.48	F^{14}_{202}

≁ Homogeneity test

* $.01 < P < .05$

** $.001 < P < .01$

significantly heterogeneous between progeny groups (Table 35). The 1964 analysis indicates that the differences in fat percentage between transferrin types AD and A; D and A; and between animals with and without the $\underline{Tf}^A$ allele, are significant. The mean fat percentage difference between transferrin D and transferrin A cows was -0.37 and between transferrin AD and transferrin A was -0.18. It should be noted that the three differences estimated (D_1, D_2 and D_3) are not additive. That is, the difference between A and AD (0.18) and the difference between AD and D (0.08) is not equal to the difference between A and D (0.37). This is due to the fact the populations on which the three differences were determined were not the same. That is, D_1 was estimated from a population of only A and AD cows, D_2 from a population of only D and AD cows, and D_3 from a population of only A and D cows. Thus the progeny groups involved and the number of animals in each progeny group varied with each estimate. The difference in fat percentage between cows with and without the $\underline{Tf}^D$ allele in 1964 was -0.22. Each of these differences showed significant heterogeneity between progeny groups.

2. Linear correlation coefficient and multiple linear regression analysis

In Table 36 are set out the results of analyses determining linear correlation coefficients for all pairs of eleven variates consisting of the observed transferrin type and ten pro-

TABLE 36

LINEAR CORRELATION COEFFICIENTS OF ALL PAIRS OF ELEVEN VARIATES: OBSERVED **TRANSFERRIN** TYPE AND ESTIMATES OF TEN PRODUCTION PARAMETERS

(a) Jersey

	Transferrin type	Fat %	Fat Yield	Milk Yield	Lactation Length	Calving Interval	Month Calving	Age	Herd	Progeny Group	Number in Herd
Transferrin Type		0.133	0.042	-0.015	-0.022	0.067	-0.026	-0.016	0.067	0.058	-0.064
Fat %	0.147*		0.402***	0.042	0.119	-0.077	-0.084	-0.012	0.467**	0.371**	-0.122
Fat Yield	0.078	0.321***		0.920***	0.487**	0.267**	0.019	0.340**	0.565**	0.674**	-0.131
Milk Yield	0.029	-0.015	0.935***		0.512**	0.314**	0.047	0.344**	0.432**	0.570**	-0.086
Lactation Length	0.053	0.036	0.552**	0.568**		-0.023	0.022	0.019	0.070	0.165*	-0.005
Calving Interval	0.069	0.022	0.279**	0.283**	-0.008		0.094	0.599**	-0.021	0.146*	0.030
Month Calving	0.017	-0.072	-0.092	-0.074	-0.127	0.182**		0.130	-0.124	-0.078	0.095
Age	0.030	-0.007	0.301**	0.313**	0.056	0.614**	0.160**		0.173**	0.340**	-0.058
Herd	0.128	0.288**	0.538**	0.461**	0.140	0.024	-0.206**	0.077		0.841**	-0.125
Progeny Group	0.110	0.279**	0.605**	0.527**	0.173**	0.143*	-0.125	0.294**	0.832**		-0.192**
Number in Herd	-0.082	-0.168**	-0.122	-0.077	0.006	-0.053	0.111	-0.087	-0.260**	-0.195*	

Above diagonal - 1963 based on 420 cows
Below diagonal - 1964 based on 486 cows

(b) Friesian

Transferrin Type		-0.219*	-0.013	0.091	0.041	-0.056	0.045	-0.060	0.301**	0.146	0.149
Fat %	-0.119		0.260*	-0.178*	0.006	-0.159*	-0.026	-0.121	0.155*	0.067	-0.220*
Fat Yield	0.104	0.257*		0.881***	0.218*	0.314**	0.025	0.452**	0.461**	0.621**	0.168*
Milk Yield	0.165*	-0.156*	0.906***		0.211*	0.408**	0.042	0.508**	0.406**	0.594**	0.283*
Lactation Length	-0.064	-0.047	0.454**	0.473**		-0.172	0.100	-0.136	0.161	0.143	0.100
Calving Interval	0.042	-0.063	0.354**	0.381**	0.013		-0.051	0.690**	-0.095	0.182*	-0.046
Month Calving	-0.027	-0.176*	0.069	0.136	-0.077	0.284**		0.030	-0.011	-0.089	0.113
Age	0.001	-0.134	0.358**	0.426**	-0.021	0.514**	0.319**		0.025	0.301**	0.021
Herd	0.304**	0.317**	0.562**	0.449**	-0.026	0.152	-0.079	0.195*		0.743**	0.338**
Progeny Group	0.272*	0.169*	0.638*	0.590**	0.087	0.278**	-0.015	0.315**	0.890***		0.261*
Number in Herd	-0.107	-0.234*	-0.094	0.005	-0.018	0.035	0.132	0.070	-0.312**	-0.171*	

Above diagonal - 1963 based on 207 cows; Below diagonal - 1964 based on 217 cows.

TfA = 0.0
TfAD = 1.0
TfD = 2.0

* $.01 < P < .05$
** $.001 < P < .01$
*** $P < .001$

duction parameters.

Transferrin type was significantly correlated with fat percantage in the 1964 Jersey data and the 1963 Friesian data, however the correlation was negative in the case of Friesian cows.

Consideration of the Friesian results also reveal significant correlations between transferrin type and herd level in 1963 and 1964 and between transferrin type and progeny group level in 1964. This can be interpreted in the sense that transferrin type D cows are concentrated in herds and progeny groups of high average fat yield.

The consistency of correlations between variates from one year to the next is apparent from the symmetry of the tables with respect to the distribution of significant correlations. Also, in the case of the Friesian breed there are no significant correlations between transferrin type and production parameters that are not attenuated by significant correlation between the production variates *per se*. For example, in 1963 transferrin type was significantly correlated with milk yield and herd level, these parameters themselves being correlated. Similarly with transferrin type, milk yield and herd level in 1964.

Table 37 presents the partial regression coefficients determined on the Jersey and Friesian data. There are no transferrin type partial regression coefficients that are significantly different from zero in the case of the Jersey breed. However, for

TABLE 37

PARTIAL LINEAR REGRESSION COEFFICIENTS AND COEFFICIENTS OF MULTIPLE CORRELATION FOR FOUR DEPENDENT AND SEVEN INDEPENDENT VARIETIES

(a) Jersey

Data	Dependent Variables	Independent Variables							R^2
		Trans-ferrin Type	Age	Calving Interval	Month Calving	Herd Level	Progeny Group Level	Number in Herd	
1963	Fat %	0.0806	-0.0259	-0.0018	-0.0010	-0.0038**	-0.0003	-0.0022	0.2416
420 Cows	Fat Yield	- 1.1768	1.4945	2.8952**	1.9672	0.1521	0.8199**	-0.1421	0.4888
	Milk Yield	-15.8227	1.9817	6.4665**	3.7860	-0.0826	1.4678**	0.0391	0.3872
	Lactation Length	- 0.0328	-0.0265	-0.0128	0.0100	-0.0043*	0.0067**	0.0028	0.0542
1964	Fat %	0.0829	-0.0361	0.0047	-0.0019	0.0009	0.0016	-0.0039	0.1160
486 Cows	Fat Yield	- 0.5413	4.2686	3.7986**	-1.8707	0.4314*	0.6660**	0.2154	0.4181
	Milk Yield	-12.1787	12.9158	6.3990**	-3.3161	0.7608*	0.9315**	0.8018	0.3399
	Lactation Length	0.0864	0.0450	-0.0107	-0.0516	-0.0006	0.0034	0.0049	0.0481

(b) Friesian

1963	Fat %	- 0.1869**	-0.0134	-0.0073	0.0022	0.0023**	0.0003	-0.0084**	0.2045
207 Cows	Fat Yield	-19.0599	17.5330**	1.2791	2.2854	0.3124	0.6356**	0.0340	0.4871
	Milk Yield	5.8660	44.8835*	7.6758	5.6589	0.2664	1.6128**	2.8529*	0.5131
	Lactation Length	- 0.0032	-0.0202	-0.0070	0.0019	-0.00004	0.0009	0.0010	0.0682
1964	Fat %	- 0.1727**	-0.0429	0.0050	-0.0117	0.0051**	-0.0025*	-0.0037	0.2499
217 Cows	Fat Yield	-11.7789	7.3142	3.4630	0.1594	0.2244	0.7713**	0.1899	0.4549
	Milk Yield	19.6781	32.7154*	6.8232	2.5967	-0.8180	2.7447**	1.6457	0.4446
	Lactation Length	- 0.1101	-0.0558	-0.0005	-0.0239	-0.0097**	0.0097**	-0.0082	0.0868

* $.01 < P < .05$

** $.001 < P < .01$

both the 1963 and 1964 Friesian data the negative partial regression of transferrin type on fat percentage is significant. Although these regression coefficients are significantly different from zero, since the seven indepedent variables account for only 20-25 per cent of the variation in fat percentage, the contribution of transferrin type to the determination of fat percentage must be regarded as minor. However, one can calculate a standard partial regression coefficient which is the regression coefficient corrected by the ratio of the variation attributable to the given variate to that of the dependent variate, i.e. $b_i' = b_i \sqrt{\Sigma x_i^2} / \sqrt{\Sigma y^2}$. These standard partial regression coefficients may then be compared directly; Table 38. It can be seen that in the Friesian population transferrin type is relatively important in its contribution to the variance of fat percentage compared with the other independent variates.

3. Simulation studies

A comparison of the means of production parameters for the different transferrin types (Tables 33 and 34, p. 104) indicated a significant effect of transferrin type on fat percentage in both Jersey and Friesian cows in 1963 and 1964, and on milk and fat yield in Friesian cows in 1964. These generalizations were not supported by the Neimann-Sorensen and Robertson (1961) analysis which indicated that only the effects on fat percentage in Friesian cows were true effects detectable within progeny groups. The

TABLE 38

ESTIMATES OF THE STANDARD PARTIAL REGRESSION COEFFICIENTS FOR THE DEPENDENT VARIATE FAT PERCENTAGE AND SEVEN INDEPENDENT VARIATES

Breed	Year	Transferrin Type	Age	Calving Interval	Month Calving	Herd Level	Progeny Group Level	Number in Herd
Jersey	1963	0.0982	-0.0752	-0.0201	-0.0054	0.4939	-0.0348	-0.0637
	1964	0.1055	-0.1119	0.0494	-0.0107	0.0901	0.1965	-0.1030
Friesian	1963	-0.2894	-0.0527	-0.1061	0.0161	0.3725	-0.0546	-0.2935
	1964	-0.2480	-0.1608	0.0550	-0.0826	0.7246	-0.4254	-0.1027

reasons for this are apparent when one examines Table 39 which gives the results of an analysis of variance in which transferrin types within progeny groups have been assigned at random. The significant variance ratio for fat percentage was not found after rearrangement of transferrin types in the 1963 Jersey data, indicating that it was the exact arrangement of the transferrin types within the progeny groups that resulted in a significant difference in the performance of transferrin types. Similarly with the fat percentage analysis for the 1963 and 1964 Friesian data. In contrast with these results are those for fat percentage in the 1964 Jersey data and for fat and milk yield in the 1964 Friesian data. The significant between transferrin types variance ratios for these three sets of data are not dependent upon the precise association of certain transferrin types with particular cows, but rather upon the concentration of certain transferrin types in particular progeny groups or herds. Thus the linear correlation analysis indicates that transferrin type D Friesian cows were concentrated in herds of high average fat yield.

The simple simulations carried out in association with the analysis of variance which takes into account the progeny group structure of the population thus provide information about the true nature of the differences between the mean performance of transferrin types. That is not to say that a two-way classification analysis involving weighting according to the numbers in subclasses

TABLE 39

ANALYSIS OF VARIANCE, TWO WAY CLASSIFICATION INTO PROGENY GROUPS AND TRANSFERRIN TYPES VARIANCE RATIOS BEFORE AND AFTER RANDOMIZATION OF TRANSFERRIN TYPES.

	Jersey		Friesian			
	1963	1964	1963	1964		
	Fat %	Fat %	Fat %	Fat %	Fat Yield	Milk Yield
Between Transferrins Variance Ratios ⌿	6.72**	7.67***	8.22***	3.16*	4.12*	9.41***
Variance Ratios after within group randomization $	1.14	3.42*	2.59	2.59	6.50**	7.61***

⌿ Extracted from Table 29

$ These are means of 3 variance ratios in 3 consecutive simulation runs

* $.01 < P < .05$

** $.001 < P < .01$

*** $P < .001$

would not give the same result as the Neimann-Sorensen (1961) analysis.

Table 40 summarizes the significant findings on transferrin type in relation to fat percentage, fat yield and milk yield.

b. Performance data analysed with respect to the J blood group

Estimates of the differences in performance between cows within progeny groups with respect to the presence and absence of J substance in the serum are given in Table 41. There are no significant differences between the two phenotypes in any of the four sets of data. The occurrence of a single significant variance ratio in twentyeight estimates is to be expected by chance.

TABLE 40

SUMMARY OF SIGNIFICANT ASSOCIATION OF TRANSFERRIN TYPE WITH PRODUCTION PARAMETERS

	Means of transferrin types			Analysis of variance, single classification				Analysis of variance two way classification		Combined estimate of difference within groups			Correlation and regression		
								VR							
	A	AD	D	VR	AD-A	D-A	D-AD	Actual	Simulated	AD-A	D-A	D-AD	r	b	R^2
Fat %															
1963 Jersey	5.228	5.335	5.449	3.78*	0.107	0.221**	0.114	6.72**	1.14	0.037	0.205	0.103	0.133	0.133	0.081
1964 Jersey	5.252	5.378	5.477	5.37**	0.126*	0.225**	0.099	7.67***	3.42*	0.056	0.129	0.062	0.147*	0.147*	0.083
1963 Friesian	3.929	3.761	3.649	5.23**	-0.168*	-0.280	-0.112	8.22***	2.59	-0.088	-0.359**	-0.124	-0.219*	-0.219*	-0.187**
1964 Friesian	3.934	3.803	3.770	1.82	0.131	-0.164	-0.033	3.16*	2.59	-0.184*	-0.367**	-0.077	-0.119	-0.119	-0.179**
Fat Yield															
1963 Jersey	377.40	369.51	397.13	1.68	-7.89	19.73	27.62	2.88	0.75	-17.04	17.91	24.47	0.042	0.042	-1.177
1964 Jersey	373.76	380.62	402.78	1.71	6.85	29.02	22.17	2.41	3.55*	- 6.47	9.51	16.19	0.078	0.078	-0.541
1963 Friesian	436.46	451.78	430.09	0.66	15.32	-6.38	-21.69	1.06	1.65	-14.51	-61.03	-33.27	-0.013	-0.013	-19.060
1964 Friesian	390.48	436.42	426.19	2.57	45.94*	35.71	-10.23	4.12*	6.50**	-21.58	-41.89	-12.81	-0.104	0.104	-11.779
Milk Yield															
1963 Jersey	721.61	692.43	724.76	1.33	-29.19	3.14	32.33	1.95	1.27	-40.01	4.98	31.41	-0.015	-0.015	-15.823
1964 Jersey	712.33	707.00	736.43	0.62	- 5.34	24.10	29.45	0.79	2.59	-22.75	0.66	25.37	0.029	0.029	-12.179
1963 Friesian	1113.47	1199.01	1186.10	1.56	85.53	72.62	-12.91	2.47	4.02*	-20.67	-48.41	-45.35	0.091	0.091	5.867
1964 Friesian	988.96	1133.51	1133.51	5.82**	171.35***	144.55*	-26.81	9.41***	7.61***	- 8.21	- 0.92	-21.52	0.165	0.165	19.678

* $.01 < P < .05$

** $.001 < P < .01$

*** $P < .001$

TABLE 41

COMBINED ESTIMATE OF THE DIFFERENCE BETWEEN J^s AND j^a WITH STANDARD ERRORS AND HOMOGENEITY TESTS

Breed	Year	No. Cows	No. Groups	Fat %			Fat Yield			Milk Yield			Lactation Length			Calving Interval			Month Calving			Age		
				Mean	S.E.	VR+	Mean	S.E.	VR+	Mean	S.E.	VR+	Mean	S.E.	VR+	Mean	S.E.	VR+	Mean	S.E.	VR+	Mean	S.E.	VR+
Jersey	1963	420	35	0.023	0.045	1.02	7.16	9.09	0.80	7.52	16.93	0.85	0.031	0.113	2.20	-0.146	0.597	0.97	0.028	0.320	1.24	-0.105	0.119	1.03
Jersey	1964	486	36	0.102	0.043	0.89	19.16	9.23	0.86	23.19	17.10	0.80	0.257	0.124	1.36	-0.190	0.499	0.72	-0.026	0.279	1.10	-0.100	0.115	0.89
Friesian	1963	207	14	-0.024	0.064	0.95	- 2.74	16.12	0.64	3.05	40.38	0.44	0.023	0.059	0.52	-0.422	0.894	0.92	-0.496	0.537	0.82	-0.219	0.232	0.71
Friesian	1964	217	15	-0.041	0.066	0.32	19.09	15.45	2.23	65.60	38.74	2.30	0.149	0.128	13.3*	0.131	0.696	0.97	0.506	0.564	0.66	0.039	0.225	0.58

+ Variance Ratio = $F_{\substack{\text{Groups} - 1 \\ \text{Cows} - \text{Groups}}}$

* $.01 < P < .05$

IV. DISCUSSION

a. Risk of bias in the data

Very large domestic animal populations are usually composed of a number of subpopulations, each having a relatively small number of individuals, with the number of males small compared with the number of females. The population structure thus facilitates the development of small groups of animals within which groups the frequency of genes at particular loci may vary by chance and therefore exhibit genetic drift. An animal group tends therefore, to have its own particular gene frequencies at any given locus and this is likely to be significantly different by chance from the gene frequencies in other groups. Since the quantitative yield characters in the animal groups vary according to management practices and the district in which they are located, there is a distinct possibility that associations may arise between certain genes and quantitative traits that are independent of the heritability of the traits. It is apparent therefore, that investigations into possible associations between single genes and yield traits in animals like dairy cattle should take into account this fact that the cattle are organized into herds, between which there is a restricted gene flow.

As pointed out by Neimann-Sorensen and Robertson (1961), spurious associations between blood-group genes and production traits can also have a genetical basis. With the growing interest in artificial breeding by insemination and the related problems of assessing the genotypic value of a sire for a production trait from the performance of his daughters, there is a tendency to regard cattle populations as composed of a number of half-sib progeny groups rather than herds. Thus, if a population includes a relatively low number of half-sib progeny groups, the chance selection of one or more groups with a relatively high frequency of a particular blood-group tansmitted by the sire and which are also composed of the daughters of a sire with a high genetic value for a production trait, an association could arise quite independent of any direct effect of blood-group genes on production particularly if the blood-group locus was linked with loci whose alleles may determine a large proportion of the variance of a production trait. Traits with high heritability such as fat percentage are more likely to show associations with blood-group genes for this reason.

linkage
not
necessary

Because of the above two problems peculiar to domestic animal populations, standard statistical procedures applied to the detection of associations between blood groups and production traits may be misinterpreted. Thus, it would seem desirable to analyse data collected by herd sampling on a within-herd basis. Although this overcomes between-herd differences as a source of between-blood-group variance, it may still confound between blood-group differences by cutting across large half-sib families. Consequently, the between-group variance can again be overestimated due to apparent associations of the genetic type discussed above. Data collected by half-sib progeny group sampling can be analysed within progeny groups to eliminate between-progeny-group variance as a significant source of between-blood-group variance. However, if the members of a progeny group are distributed over a number of herds in several districts, this could lead to over estimation of the between-blood-group variance for non-genetic reasons discussed previously. Thus it is desirable that any investigation into blood groups and production traits should involve sampling of half-sib progeny groups within farms. Such a sampling method allows the valid use of a within half-sib progeny group analysis for the detection of real effects of blood-group genes.

It should be stressed that other forms of analysis such as between progeny group analysis, given the blood-group of the sire, whilst relatively insensitive for the detection of blood group effects (Neimann-Sorensen and Robertson, 1961) and prone to the detection of spurious associations, can reveal true associations and Rendel (1959, 1961) in two different samples found the same association that has since been confirmed using within-progeny-group forms of analysis (Neimann-Sorensen and Robertson, 1961; Conneally, 1962; Conneally and Stone, 1965).

within sire method

b. The effect of transferrin type on production in dairy cattle

From the above discussion it is apparent that the significant associations tabulated in the results of Section B could be due to one or more of the following causes:

When a large number of significance tests are carried out simultaneously, at the 5% level of significance, 5% of the deviations from expectation should be significant. In the case of the analyses reported in this thesis, the frequency of significance tests is higher than that expected if all significant results were due to chance alone.

Because most of the progeny groups were on different farms, the genetic and environmental components of the between-progeny-group variance cannot be separated, thus both genetic and environmental factors could account for the apparent associations detected between transferrin type and fat percentage in Jersey cows using single classification analysis of variance, simple correlation, and analysis of variance two-way classification. Similarly for Friesian cows and milk and fat yield (Table 40). This conclusion is based on the observation that the within-progeny-group analysis showed no significant differences in any of these cases.

The within-progeny-group analysis indicates that the Friesian sample shows a real association between transferrin type and fat percentage. That is, transferrin A cows had a significantly higher fat percentage than transferrin D cows for both years 1963 and 1964.

In contrast with the Jersey results too is the observation that the partial regression of fat percentage on transferrin type is significantly different from zero for both years. Thus, if multiple linear regression analysis had been used alone on all data the conclusions drawn would not have been incorrect as shown by the within-progeny-group analysis. In this connection it is of interest that the analysis of Ashton *et al.* (1964) was a multiple linear regression analysis. These workers found that in Jersey and A.I.S. cows, transferrin D animals produced more milk per year than transferrin A animals. The negative correlation generally found between fat percentage and milk yield (Johansson, 1961) might lead one to expect therefore that in the same sample there may be an effect of transferrin type on fat percentage but in the opposite sense. Indeed, there is some indication of this in their Jersey results (Figure 21, p. 92). The results for Friesian cows are, therefore, not incompatible with those of Ashton *et al.* (1964) since in the Friesian population there was a significant correlation between milk yield and fat percentage (Table 36). The absence of a significant correlation between milk yield and fat percentage in the Jersey sample (Table 36) is interesting in view of the fact that there was no significant effect of transferrin type on fat percentage in these animals.

In Table 35 there are numerous significant variance ratios (homogeneity tests) indicating that the production differences between genotypes varies with progeny group. These are manifestations of the hypothetical problems discussed initially, i.e. the effect of single gene differences on quantitative traits depends upon the genetic and physical environments of the animals and their organization into groups.

Failure to find a significant effect of transferrin type on milk yield in either population is a different result from that obtained by Ashton _et al_. (1964). There are a number of possible reasons for this:

(i) The truncated distribution of lactation length in the South Australian sample

Since Ashton _et al_. (1964) concluded that transferrin type affected milk yield by increasing average daily yield and lactating length, the restriction of lactation length to less than or equal to ten 30-day periods in the South Australian sample (p. 97) would reduce the sensitivity of any test for an effect of transferrin type on milk yield.

(ii) Different management practices in the two States

The South Australian cows were drawn from stud herds which are maintained to sell animals on the basis of their performance or the performance of their relatives. For this reason they are generally fed in excess of those levels recognized as most economical for milk production. The mean milk production of these animals was in the general range 600-900 gallons for Jerseys and 800-1400 gallons for Friesians (Appendix III). In contrast the Queensland animals were from grade herds in the Kingaroy and Nambour regions that are primarily peanut areas and marginal for dairy farming. The mean milk production of these animals was in the general range 300-450 gallons for Jerseys and 400-600 gallons for A.I.S.

The two samples are, therefore, very different with respect to their levels of feeding management. The transferrin genotypes may not show any effect on milk yield where cows are already giving their maximum output as a result of high feeding levels.

(iii) Different genetic backgrounds of the transferrin loci

The Queensland and South Australian samples both included large samples of cows of the Jersey breed. Since these samples were from two populations that have been genetically isolated for many years (Barker, 1957), it is possible that the genetic environment of the transferrin genes in each is different.

(iv) Different frequencies of $\underline{Tf}^{D_1}$ and $\underline{Tf}^{D_2}$

Although the frequencies of $\underline{Tf}^{D_1}$ and $\underline{Tf}^{D_2}$ would markedly affect the results obtained if the apparent effect of $\underline{Tf}^{D}$ on production was due to either one of these alone, the limited frequency data for these genes in South Australia (Section A, Table 20, p. 48) failed to show that they are significantly different from cattle populations in other parts of the world. It is unlikely therefore, that, within breed, the South Australian frequencies are significantly different from those in Queensland. However, in the absence of data on this point it remains a possibility.

It may be significant that in this study the Jersey population had a very low frequency of $\underline{Tf}^{D_1}$ (0.08 see Table 20, p. 48) and did not show the association between fat percentage and transferrin type whereas the frequency of $\underline{Tf}^{D_1}$ in the Friesian population was 0.47. It is thus tempting to postulate that the apparent effect of the $\underline{Tf}^{D}$ "allele" in depressing fat percentage may actually be an effect of the $\underline{Tf}^{D_1}$ allele.

From the above discussion it can be concluded that whilst the results obtained in this study are not incompatible with those of Ashton _et_ _al_. (1964), they are different for unknown reasons and a number of possibilities have been proposed. Recently, workers in Werribee, Victoria, (White and Banfield, 1967) have shown that in 378 cows in this area there was a significant difference in milk production between transferrin types (D > A) but since there was an inverse relationship with fat percentage (A > D), the actual butterfat yields were not significantly different. This finding confirms that described in the Friesian cows in this thesis.

c. The use of computer simulation as an aid to statistical analysis

Simple simulations of the type used in this study are useful for examining further, apparent associations that have been found using various forms of statistical analysis. In this study the within-progeny-group randomization of animals with respect to transferrin types in the two-way classification analysis of variance indicated that the associations found in Jersey cows were probably spurious (Table 39, p. 110). In the within-progeny-group analysis the results supported the suggestion that it was the between-progeny-group variance that was responsible for the association between fat percentage and transferrin type in Jersey cattle, and the fat and milk yield and transferrin type associations in Friesians.

d. The contribution of transferrin type to the variance of fat percentage

Ashton (1960b) found that the difference between the mean genetic value of transferrin A cows and transferrin D cows was 50 gallons of milk (D > A), and since the range of contemporary comparison values in his population of bulls was -130 to +160 gallons or approximately 300 gallons of milk, he claimed that the transferrin locus was responsible for 17 per cent of the total genetic variation in milk yield in the major milk breeds. In a later study Ashton et al. (1963, 1964) estimated that the transferrin locus was responsible for 6.0 - 10.4 per cent of the genetic variation in milk yield (p. 93).

From estimates of the standard partial regression coefficients (b') presented in Table 38 (p.109) it may be inferred that transferrin type in the Friesian population is relatively important in its contribution to the variance of fat percentage relative to the other independent variables examined. That is, only herd level, progeny group level and number in herd had higher values of b' than transferrin type. It would be useful therefore to determine the proportion of the genotypic variance of mean annual fat percentage that is attributable to the transferrin locus. However, since the estimates of the difference in fat percentage between transferrin types are not additive (see p.107), the genotypic values adopted for the three transferrin types would be arbitrary. Also, since satisfactory heritability estimates cannot be derived from the data due to the fact that the half-sib-families are located in a number of different herds and the numbers of animals in the families are relatively low, values for the heritability of fat percentage would have to be adopted from the results of other workers. Thus, any estimate of the proportion of the genotypic variance of fat percentage attributable to the transferrin locus that was made from the results presented here would not be very meaningful. All that can be said is that the differences in fat percentage between transferrin types are surprisingly high as is the relative importance of transferrin type as an independent variable in the multiple linear regression analysis.

The physiological mechanism by which certain transferrin genes might affect the fat percentage of milk or the quantity of milk produced by an animal is not known. Osterhoff (1966a) could not demonstrate production superiority of cattle of any transferrin type when they were subjected to nutritional stress and Hines (1965) could find no immunological differences between transferrin types. However, Neethling and Osterhoff (1966) showed that the relative iron-binding ability of the different transferrin types was in the order A, D, AE, AD, DE, E, where A was highest and E lowest, and they presented data which they said showed a relationship between the iron-binding ability of transferrin types and the growth rate of young bulls. No statistical analysis was presented. Whatever the mechanism by which transferrin type affects production it is almost certainly not related to the mechanism maintaining the polymorphism in cattle populations, since effects on production have been found by a number of workers, especially in Australia (Ashton et al., 1963, 1964; White and Banfield, 1967; and this thesis) whereas there is little accumulated evidence for a

doubtful result indeed!

selective advantage of any transferrin genotype over another (literature survey, p. 17). The possible role of artificial selection in the maintenance of the transferrin polymorphism is discussed below (p. 124).

e. Practical implications of the observed effect of transferrin type on fat percentage

Since the average effect on fat percentage of substituting $\underline{Tf}^A$ for $\underline{Tf}^D$ in the Friesian population is 0.18 if one assumes a genotypic value for AD that is midway between A and D, then it could be argued that selection of cattle for transferrin type would result in a relatively large increase in fat percentage. However, even if this were shown to be true, the frequency of the transferrin alleles is such that the time involved in achieving homozygosity for $\underline{Tf}^A$ in a large proportion of animals on a large scale would be of the order of 20 years depending of course on the number of bulls used in each generation. Also, since this increase in fat percentage is unlikely to result in any nett increase in fat yield (White and Banfield, 1967), and in view of the trend towards payment for milk on a protein basis, it is unlikely that the apparent effect of transferrin type on fat percentage will be of any practical significance.

f. Artificial selection and the transferrin polymorphism

If selection for high fat percentage resulted in an increased frequency of the $\underline{\mathrm{Tf}}^{A}$ allele each generation then one might expect that eventually the $\underline{\mathrm{Tf}}^{A}$ allele would be fixed in the population. However, in view of the fact that in this country at least, very few animals are culled for production reasons, and even if they are they will still have produced at least one offspring, artificial selection is unlikely to have any measurable effect on the frequency of the $\underline{\mathrm{Tf}}^{A}$ allele. Whilst this is true, the observation that in populations of non-domestic cattle such as Bison, Wisent, Banteng and the African buffalo there is no transferrin variation, could indicate that in domestic cattle artificial selection may play a role in the maintenance of the transferrin polymorphism. Thus it would seem desirable in this connection to examine other traits in cattle, such as resistance to disease or internal parasites, that might be important in relation to superior fitness of transferrin heterozygotes under conditions of intensive herding of domestic cattle.

g. The effect of alleles at the J locus on dairy cattle production

No effect of the presence of J substance in the serum on production has been shown: however, since J^{cs} was not specifically tested, this observation cannot be regarded as contrary to those observations made by Rendel (1959, 1961) who found that the J^{cs} phenotype was associated with high butterfat percentages in Swedish dairy cattle.

APPENDIX I

NOTES ON TECHNIQUES AND REAGENTS

A. BOUNDARY BEHAVIOUR AT THE POINT OF SAMPLE INSERTION IN STARCH GEL ELECTROPHORESIS RUNS*

(D.W. Cooper and L.F. Bailey, 1966)

Cattle and sheep sera have been typed for transferrin in this laboratory using the discontinuous tris-citrate/lithium borate buffer system of Ferguson (see Ferguson and Wallace, 1961; Ashton and Ferguson, 1963). Samples were inserted into precast slots as described by Smithies (1959). In some runs a phenomenon which came to be described as "streaking" occurred; individual protein zones were V-shaped and concentrated at their centre while streaks of protein ran the length of the gel through the centre of the bands.

It was found that the defect of streaking was associated with the time taken for the electrolyte boundary to reach the sample slots. This time could be controlled either by using the type of gel bed described by Smithies (1959) where the front first passes through a thick cathodic block of starch or, in gel trays which did not have the end reservoirs, by placing the slots further from the cathodic end of the gel. Thus, the problem of streaking of trans-

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* Reported in Immunogenetics Letter, 4: 115-116.

ferrin bands which made typing impossible was overcome. However, it was considered of interest to determine the exact cause of streaking.

Two gels were set up which were similar in all respects except that one had the sample insertion slots set 1½" closer to the cathodic end of the gel bed than the other.

A blue marker dye, bromophenol blue, was included in the buffer when the gels were prepared (Bleomendahl, 1963) to facilitate the observation of the electrolyte boundary and the serum albumin to which it became attahced. To facilitate further observation of the process, haemoglobin was added to serum samples and a polythene film was placed over the sample slots in place of the usual vaseline.

It was found that when the electrolyte boundary reached the sample slots it travelled between them in radiating circles then rejoined on the anodic side (Figures 22, 23). Where the slots were reached in a relatively short time, the serum proteins had not moved sufficiently far to avoid interference by the irregular boundary. This did not occur if the slots were placed further down the gel. The middle of a protein zone in a streaked gel is slower than either of its ends (this is particularly marked in the slow alpha-2). One explanation of this effect is that the ends of a band enter the high voltage region behind the boundary (Poulik, 1957) before the middle.

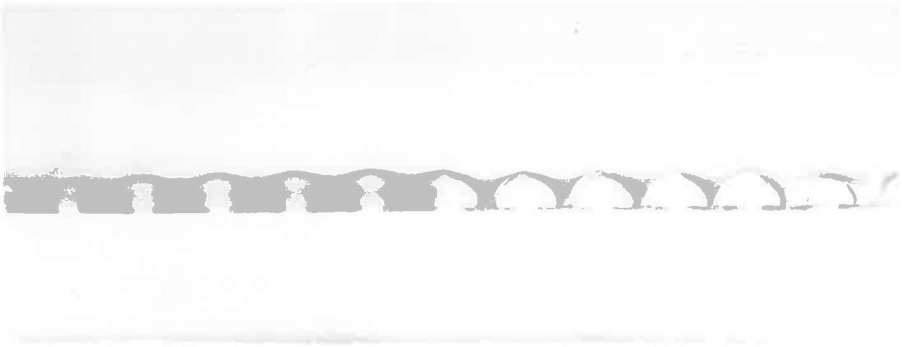
Where the filter paper method of sample insertion is used, this defect presumably could not occur. However, trials have

FIGURE 22.

Boundary behaviour when the sample slots are in a position which normally results in the production of a streaked gel.

TOP Front just past the slots. The irregular boundary is associated with the serum albumin and where haemoglobin has been added the haemoglobin band is markedly distorted.

BOTTOM Forty minutes later. The haemoglobin is obviously streaked and the albumin zones are concentrated at their centres. The albumin zones are sharp at their leading edge and diffuse at their trailing edge which is atypical albumin behaviour in this buffer system.



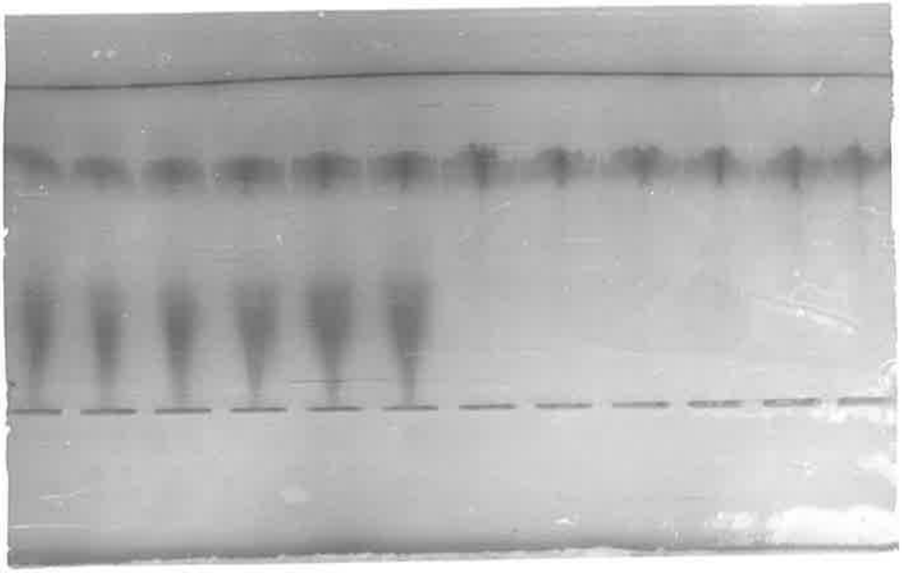
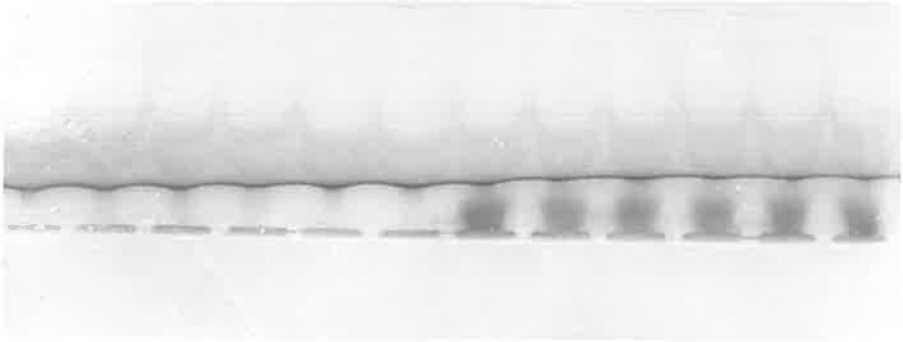
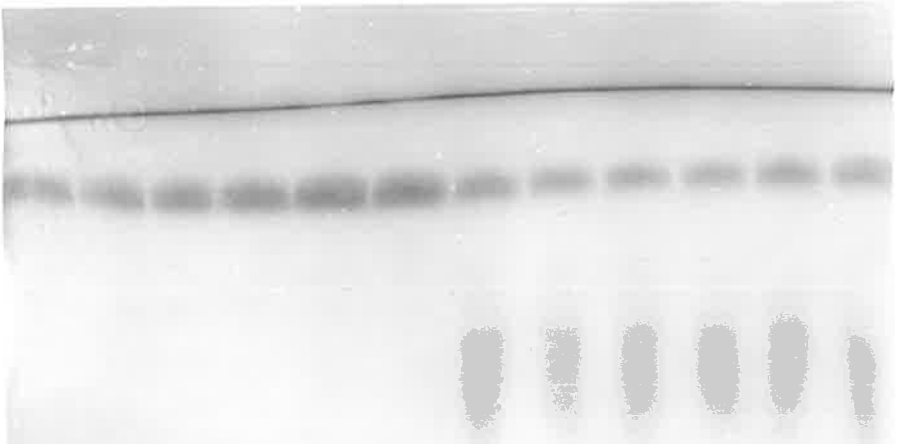


FIGURE 23.

Boundary behaviour when the sample slots are placed sufficiently far down the gel to avoid interference with the protein patterns by the irregular front.

TOP	Front just past sample slots. The albumin at least is out of range of the irregular front.
BOTTOM	Thirty minutes later. Albumin zones are characteristically sharp at their trailing edge and diffuse at their leading edge. The migrating boundary is slightly crooked.





shown that if the paper is not removed before the boundary reaches it, then "streaking" does occur.

B. A SIMPLE APPARATUS FOR PAPER ELECTROPHORESIS*

(L.F. Bailey and D.W. Cooper, 1966)

A simple apparatus for paper electrophoresis has been constructed and used in this laboratory for the large scale typing of sheep and cattle haemoglobins. It has the following advantages.

Its total cost is about $8, at least 40 samples may be typed at each run, the resolution is at least as good as with commercial baths, and the apparatus can be constructed and dismantled in about 15 minutes.

The apparatus is shown in Figure 24. It consists of two sheets of glass, each 26" x 15" x $\frac{1}{4}$", about 30 feet of fine nylon fishing line, 5 feet of $\frac{3}{4}$" and 5 feet of $\frac{3}{8}$" external diameter polythene tubing.

The $\frac{3}{4}$" tubing is placed on one of the glass sheets in the form of an ellipse and may be fixed to the glass for convenience using a commercial contact cement.

The $\frac{3}{8}$" tubing is cut into four lengths slightly shorter than the edges of the glass sheet, then each is split longitudinally and pushed over the edges of the glass to cover the sharp edges.

The fishing line is tied around the bottom sheet of glass, the polythene at the edges preventing severing of the taut line. Four lengthwise runs and two cross runs form a lattice on which rests a

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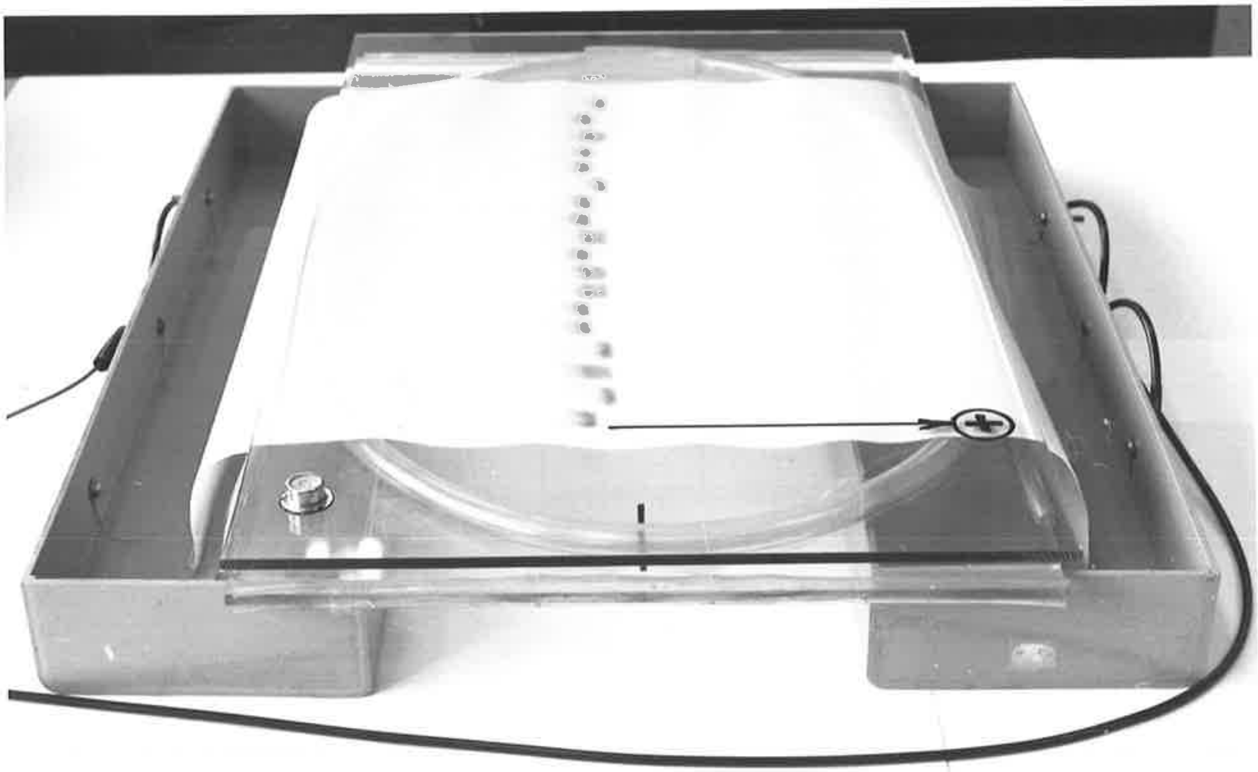
* Reported in Immunogenetics Letter, 4: 112-113.

FIGURE 24.

The paper electrophoresis apparatus showing 18 samples of cattle haemoglobin at the end of a run.



piece of Whatman 3 mm chromatography paper, 18" x 22", which has been wetted in buffer and blotted between sheets of blotting paper.

The top sheet of glass rests on the tubing and the paper, forming a good seal. The bottom sheet rests on the electrolyte trays.

Presumably, this method of forming a sealed chamber may have application to electrophoretic systems using other supporting media.

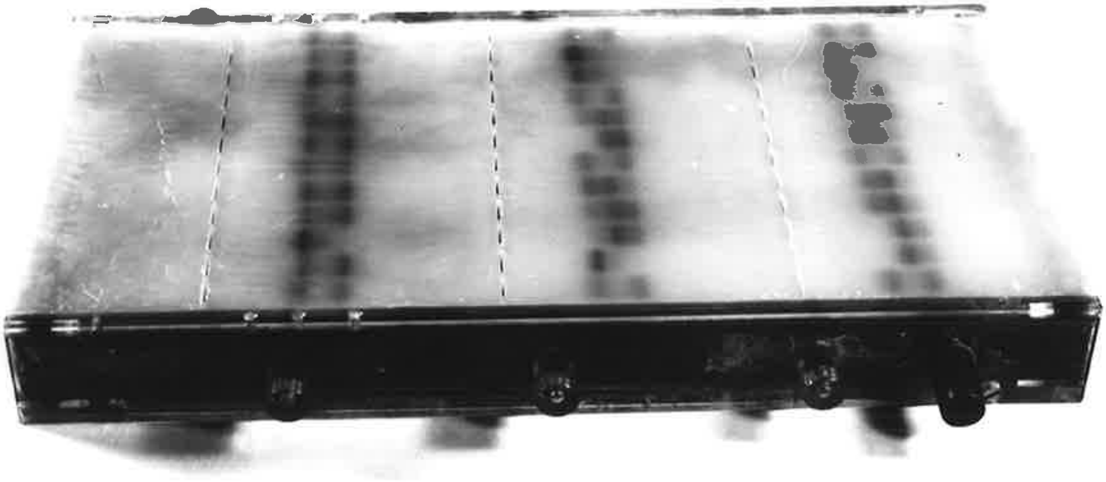
C. LARGE SCALE HAEMOGLOBIN TYPING IN STARCH GEL

Although the paper electrophoresis apparatus described above (Appendix I) can be used to type about 40 haemoglobin samples per run, paper-electrophoresis runs are relatively slow compared with electrophoresis in starch gel although the preparation of starch gels is more tedious. Because of the better resolution of haemoglobins obtained by starch gel electrophoresis and because paper-electrophoresis runs tended to be subject to mishap necessitating re-runs, the apparatus used for the electrophoresis of serum was adopted for the large scale typing of haemoglobin. This was effected by constructing a compound lid for the gel trays which could accommodate 3 slot formers.

Figure 25 shows the result of electrophoresis of 36 haemoglobin samples using the buffer system attributed to O. Smithies by Huehns and Shooter (1965). Using the four gel trays normally used for transferrin typing it was possible to type 132 samples per run, allowing 3 standards per tray.

FIGURE 25.

A gel tray containing an unstained starch gel block in which 36 haemolysates have been subjected to electrophoresis.



D. A COMPARISON OF THE ANTI-SHEEP O REAGENTS OF CATTLE AND GOATS*

(L.F. Bailey, 1967)

Naturally occurring antibodies against sheep O red cells have been found in the sera of cattle (Stormont, 1951) and goats (Suzuki and Stormont, 1961). The cattle reagent has been used to detect O-like substances in the sera of cattle (Sprague, 1958) and goats (Suzuki and Stormont, 1962) using inhibition tests and these substances have been called Oc and Og respectively.

For detecting O substance in sheep and cattle sera the author has routinely used a cattle reagent kindly supplied by Professor C. Stormont (serum number 281) which has a titre of 32 against sheep O cells when used with rabbit complement. This gives more rapid and stronger reactions than does guinea pig complement.

Because of the inconvenience of importing sera from California, a search for anti-O in goat serum was undertaken. The author, with a number of colleagues (Cooper, 1966; Alexander, Williams and Bailey, 1967) screened 100 goat sera and found anti-O in only 6. Most of these were in the range of titres 2-8, however, one animal was found with an anti-sheep O titre of 256 when used with guinea pig complement. When rabbit complement was used the titre was 64.

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* Reported in Immunogenetics Letter, 5: 81-83.

Inhibition titres of both reagents with a number of sera were determined using the same dilutions of test sera to inhibit the cattle reagent diluted $\frac{1}{8}$ and the goat reagent diluted $\frac{1}{100}$. Table 42 shows the inhibition titres obtained using 22 cattle sera and four sheep sera. Unlike different dilutions of the same reagent, the two reagents do not give parallel differences in inhibition titre. The correlation between the $\log_2$ titres obtained using the two reagents is 0.41 ($.01 < P < .05$). In general, the goat reagent gives lower titres than the cattle reagent, but in three cases the order is reversed. Although the anti-O concentration in both reagents is approximately the same, since both represent the maximum dilution that can be made without leaving unhaemolysed cells in control tests, the goat antibody is relatively insensitive to the presence of O substance in solution. Of particular interest are the observations on R sheep sera. In 82 R sheep sera tested with the cattle reagent (2 shown in Table 42) a low titre of O inhibition (1-4) was detected in 81 cases (Bailey, unpublished). No inhibition can be detected with goat reagent.

It may be concluded that either:

(1) The goat antibody is specific for a different part of the O substance from the cattle antibody and this part is obscured to varying degrees when the substance is in solution.

TABLE 42

A COMPARISON OF THE ANTI-SHEEP O REAGENTS OF CATTLE AND A SAANEN GOAT

Serum		Inhibition titre of O substance	
		Cattle anti-sheep O	Goat anti-sheep O
Cattle JOc	1	1	4
	2	1	4
Cattle Oc	1	4	2
	2	16	8
	3	16	16
	4	8	16
	5	64	8
	6	4	2
	7	32	8
	8	4	2
	9	16	1
	10	16	2
	11	16	4
	12	32	8
	13	4	1
	14	16	4
	15	32	4
	16	4	2
	17	8	2
	18	16	4
	19	8	2
	20	32	8
Sheep R	1	1	0
	2	1	0
Sheep O	1	16	4
	2	16	4

(2) The cattle reagent is sensitive to other substances as well as O substance.

If the first alternative is valid then the goat reagent is unsuitable for titring O substance in solution. Whether all samples of goat anti-sheep O behave in this manner is not known since only one animal with a substantial titre has been found.

ADDED NOTE

After this note was published, the sera of 90 cows at the Northfield Research Centre were examined for Oc substance using both reagents. Sixty were positive using the cattle reagent and 21 were positive with the goat reagent. In every case where sera were positive with goat reagent, they were also positive with the cattle reagent (30 reacted with neither reagent). When the red cells of cattle were tested for the presence of Oc substance by absorption, none were positive when the goat antibody was used and only those whose serum inhibited the goat antibody were red cell Oc positive when the cattle reagent was used. That is, there are two types of Oc reactors, those with Oc substance in their serum and on their red cells (Oc^{cs}) and those with Oc substance only in their serum ($O\overset{s}{c}$). These classifications are comparable with J^{cs} and J^{s} when J substance is considered. Although Sprague (1958a) did not report in detail the results of absorption of anti-O using red cells from Oc positive cattle, he inferred that all Oc positive cattle were Oc^{cs}. The reason for this discrepancy is not known. It is unlikely that all Sprague's cattle were Oc^{cs}.

It is apparent from these results that there are differences between the Oc substance titres of Oc positive cattle and that when the Oc titre is sufficiently high, the substance becomes absorbed into the red cell. The different results obtained with the goat and cattle reagents supports the idea that the goat reagent is relatively insensitive to the presence of Oc substance in solution and that it is specific for a different part of the Oc substance.

APPENDIX II

THE R-O-i BLOOD GROUP OF SHEEP. A MIXED R/O PHENOTYPE IN FOETUSES.

a. Introduction

In 1958(a) Sprague postulated that the exceptions to the allelic hypothesis for the inheritance of the J and Oc substances in cattle could be due to the control of the JOc blood groups by two loci, as is the case with the R-O-i blood groups of sheep. Since it was hoped to gain information on this point by studying the development of the JOc blood groups in cattle foetuses, the R and O substances in sheep foetuses were first examined because the genetic control of the R-O-i blood groups in the sheep had been established: viz. the difference between the R and O blood groups of sheep is controlled by two alleles, R and r, R being dominant to r (Kaczkowski, 1928; Andersen, 1938; Ycas, 1949; Stormont, 1951). The presence or absence of antigenicity of either type is controlled by genes I and i at a second locus where I is dominant to i and red cells of ii individuals do not react with anti-R or anti-O (Rendel, Neimann-Sorensen and Irwin, 1954; Rendel, 1957; Tucker, 1962). Both R and O are soluble specific blood-group substances found in the plasma which become coated onto the red cells. Newly-born lambs have R or O antigens in their plasma, but not on their red cells until 3-4 weeks after birth (Ycas, 1949;

Rendel, 1957).

Since R sheep have large amounts of O substance in their saliva, Rendel (1957) has suggested that the production of O substance is determined by the I gene. Further, the presence of O substance in the plasma of sheep is highly correlated with the presence of plasma alkaline phosphatase isozyme B (Rendel and Stormont, 1964; Rendel, Aaland, Freedland and Møller, 1964; Rasmusen, 1965), and since intravenous injection of O substance into an R sheep results in the rapid appearance of the B isozyme in the plasma, O substance may be a mediator of the release of B phosphatase into the plasma (Rendel et al., 1964). However, these workers also observed that most young lambs had a B phosphatase zone regardless of their R-O-i phenotype.

This appendix presents the results of a study of the R and O blood group substances and alkaline phosphatase isozymes in the serum of foetal sheep, young lambs and adult sheep, and discusses the findings in relation to the above observations. The J and Og blood-group substances of goat foetuses and adults are also considered.

b. Methods and Materials

The R-O-i and J blood groups were determined by the inhibition test described by Rendel (1957). Standard sera used for inhibition tests were: (1) cattle anti-sheep O diluted $\frac{1}{8}$ (code No. 281, C. Stormont) and (2) cattle anti-J absorbed with sheep O cells and diluted $\frac{1}{100}$.

Blood was collected from sheep foetuses at the Metropolitan and Export Abattoirs, Gepps Cross, South Australia. These came from slaughtered ewes, predominantly Australian Merino or Merino crosses. Samples were also obtained from a limited number of ewes and foetuses as well as from a ram which was the sire of the foetuses. These samples were supplied by Dr. G. Alexander of the C.S.I.R.O., Prospect, N.S.W.

Alkaline phosphatase activity was detected in starch gels by staining as described by Rendel and Stormont (1961) and Rendel *et al.* (1964).

c. Results

The results of inhibition titres carried out on foetal and other sera are shown in Table 43. All foetuses had O substance in their serum. Foetuses in the table are arranged in order of increasing crown-rump length and it is apparent that there is no obvious relationship between the age of the foetuses and the titre of R or O substances in their serum. However, those foetuses with a developed birth-coat had lower titres of both R and O substances than the younger foetuses. Lambs at four weeks of age also had low titres of R and O. NF 2 was the only lamb of type O when the red cells were tested. Adult sheep had similar titres of R and O substances depending upon whether they

TABLE 43
R-O INHIBITION TITRES AND ALKALINE PHOSPHATASE TYPES OF SHEEP FOETUSES, LAMBS AND ADULTS TOGETHER WITH A SIMILAR STUDY ON GOATS.

	Length (ins.)	Sex	R Substance	O Substance	Phosphatase A	Phosphatase B
	2¾	M	16 4	32 4	+	−
	3½	M	16 4	8 3	−	−
	3¾	M	16 4	32 5	+	+
	4¼	F	4 2	8 3	+	+
	"	F	2 1	16 4	+	+
	"	M	2 1	16 4	+	+
	"	M	4 2	4 2	+	+
	"	F	2 1	32 5	+	+
	"	F	2 1	8 3	+	−
	"	F	4 2	16 4	+	−
	"	M	1 0	1 0	−	−
	"	M	0 0	32 5	+	+
	4¾	F	8 3	1 0	+	−
	5	M	8 3	4 2	+	+
	"	F	256 8	128 7	+	+
	"	F	128 7	8 3	+	+
	"	F	64 6	8 3	+	+
	5¼	M	16 4	4 2	+	+
	"	F	32 5	16 4	+	+
	"	M	1 0	4 2	+	+
	"	F	2 1	8 3	+	−
	"	F	32 5	4 2	+	+
	"	M	2 1	1 0	+	−
	6	F	64 6	4 2	+	−
	6¼	F	1 0	2 1	+	−
	6½	M	2 1	2 1	+	+
	6¾	M	0 0	64 6	+	+
	"	M	0 0	8 3	+	−
	"	F	1 0	8 3	+	−
	7	F	32 5	8 3	+	+
	7½	M	2 1	4 2	+	−
	"	F	16 4	4 2	+	−
	"	M	1 0	8 3	+	+
	"	F	1 0	1 0	+	+
	7¾	M	0 0	1 0	+	+
	8	M	16 4	256 8	+	+
	"	F	64 6	8 3	+	+
	"	F	32 5	4 2	+	+
	"	F	2 1	4 2	+	+
	8¼	M	16 4	1 0	+	−
	8½	M	16 4	2 1	+	+
	"	F	2 1	8 3	+	+
	"	F	1 0	16 4	+	+
	"	M	2 1	1 0	−	−
	"	F	1 0	1 0	+	−
	9½	M	0 0	16 4	+	−
	"	F	1 0	1 0	+	−
	10	F	4 2	32 5	+	−
	"	F	4 2	4 2	+	+
	10½	M	2 1	4 2	+	−
	11½	F	1 0	16 4	+	+
	"	F	32 5	16 4	+	+
	12½	M	32 5	16 4	+	+
	"	F	128 7	512 8	+	+
	12¾	F	16 4	8 3	+	+
	13	M	2 1	64 6	+	+
	13½	F	16 4	8 3	+	+
Foetuses with Birthcoat	14½	F	8 3	16 4	+	+
	"	F	0 0	2 1	+	−
	15½	M	0 0	1 0	+	−
	15	M	2 1	8 3	+	+
	15	F	0 0	8 3	+	+
	15½	M	1 0	1 0	+	−
	15	M	2 1	2 1	+	+
	15½	M	2 1	8 3	+	+
	18½	M	2 1	2 1	+	+
	19¼	M	0 0	8 3	+	+
	−	M	2 1	1 0	+	+
	−	F	1 0	2 1	+	+
Lambs, 4 weeks	NF1	−	4	1	+	+
	NF2	−	0	4	+	+
	NF3	−	2	1	+	+
	NF4	−	2	1	+	+
	NF5	−	2	1	+	+
Ewes	S19	M	0	32	+	+
	S21	F	8	0	+	−
	GDB	F	16	2	+	−
Goat Foetuses	6	M	0	1		
	6	F	0	0		
	7½	M	0	0		
	"	F	0	0		
	"	M	8	16		
	"	M	8	32		
	"	F	4	2		
	8½	F	0	0		
	19	−	0	0		
Does	WPD	F	0	1		
	AN5	F	16	2		
	AN1	F	32	0		
	AN10	F	32	0		

Pairs of individuals bracketed together represent twins.

Three individuals bracketed together represent a triplet.

were blood group R or O. Although most adult sheep had low titres of O substance (81/82) no O sheep had similar low titres of R substance (82 tested, 3 shown in Table 43).

Table 44 shows the number of foetuses in the four phenotypic classes R, O, R/O and i.

Figure 27 shows the $\log_2$ titres of O and R substances in the sera of RO sheep foetuses. There is a significant positive correlation between the titres of the two substances. However, if the three sera with high titres of both R and O substances are omitted from the analysis, the correlation disappears.

When 0.08 ml foetal serum was subjected to starch gel electrophoresis as described by Gahne (1963a), a fast zone (the A zone) of phosphatase activity was found in all but three of the 69 samples. These three showed no phosphatase activity at all. Of the remaining 66, 47 also showed a second zone (the B zone). Phosphatase staining in the B zone was never intense and usually faded rapidly when the gel was washed in methanol. In general, foetuses without B zone activity had less A zone activity than those with a B zone. Figure 26 shows a diagrammatic representation of the phosphatase patterns obtained in foetuses, lambs and adults. All five lambs listed in Table 43 had phosphatase B although NF 2 is the only lamb of blood group O. The B phosphatase zone observed in these lambs was slightly faster in mobility than that of adults which was in turn faster than the

TABLE 44

NUMBER AND FREQUENCY OF FOETUSES IN THE FOUR INHIBITION CLASSES AND THOSE EXPECTED ASSUMING VARIOUS CAUSES FOR THE R/O PHENOTYPE AND VARIOUS GENE FREQUENCIES.

Details	Inhibition Type R	O	R/O	i	Total	χ^2_1
Observed number in classes assuming all titres valid	0	9	60	0	69	
Frequency	0.00	0.13	0.87	0.00	1.00	
Expected number in classes assuming all R/O foetuses are I-R- and q = 0.6	0	25	44	0	69	16.1 $P < 0.001$
as above, q = 0.5	0	17	52	0	69	5.00 $0.01 < P < 0.02$
Expected numbers in classes assuming R/O foetuses are both I-R- and due to placental transfer q = 0.6	0	15	54	0	69	3.07 $.05 < P < .1$
as above q = 0.5	0	8	61	0	69	0.14 $.7 < P < .8$
Observed numbers in classes disregarding titres of 1	5	13	44	7	69	

q = frequency of the <u>r</u> gene

FIGURE 26.

A diagrammatic representation of the alkaline phosphatase patterns of foetuses, lambs, and adult sheep.

ALKALINE PHOSPHATASE ISOZYMES IN SHEEP SERUM

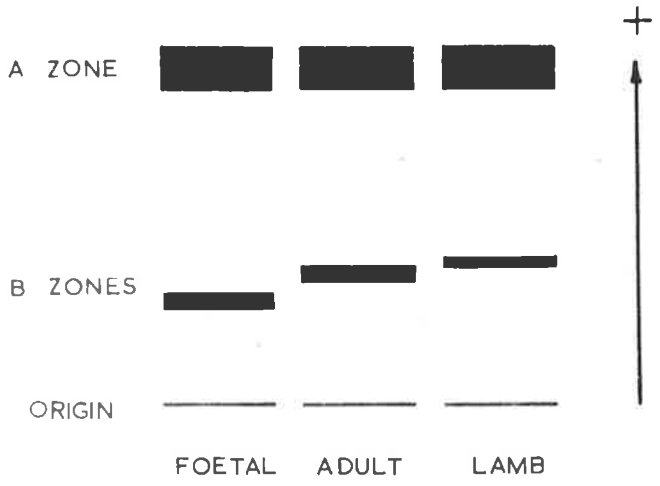
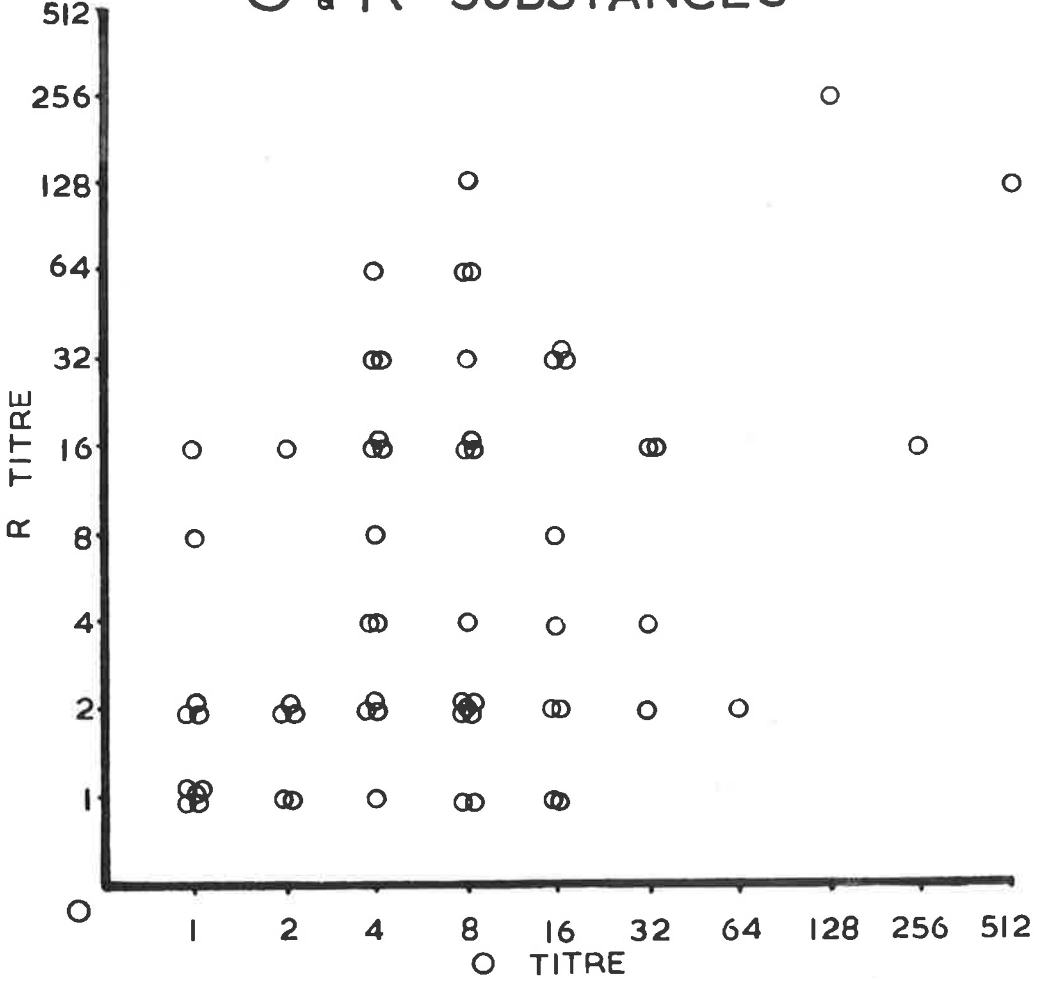


FIGURE 27.

The titre of O and R substances in the sera of sheep foetuses of inhibition type R-O. The $\log_2$ titres are significantly correlated, $r = 0.325$ ($.01 < P < .05$). If the three sera with the highest O titre are omitted from the analysis, $r = 0.09$ which is not significant.

O & R SUBSTANCES



B zone of foetuses.

In Table 45 family data is presented for the inheritance of R-O-i blood groups where the offspring of matings are tested as foetuses. Ten ewes were all mated to the same ram and the foetuses removed at 60 days. A further 11 incomplete families are presented, these being derived from dam-foetus pairs collected at the abattoirs. With a single exception, all O ewes carried O foetuses and all R ewes carried RO foetuses.

d. Discussion

The observation that most sheep foetuses have both R and O substances in their serum has a number of possible explanations.

1. Technique

It could be argued that since some R sheep show low titres of anti-O inhibition possibly due to non-specific inhibition of anti-O by R substance, some of those foetuses classified as R/O are actually R or O, the low titres of the antithetical antigens being artifacts. If one considers all titres of 1 as artifacts the resultant classification of the 69 foetuses is given in Table 43. This results in the transfer of 5 individuals to the R class, 4 to the O class, and 7 to the i class. A large proportion however, remain in the R/O class. The absence of both antigens is not related to the age of the foetus and this manipulation has produced a class not previously described in the Australian Merino

TABLE 45

R AND O SUBSTANCES IN FOETAL SHEEP SERUM - FAMILY DATA

Sire	Dam	Foetus
R	O	O
(R/r)*	O	O
	O	O
	O	O
	R	R-O
	R	O
	R	R-O
	R	R-O
	R	R-O
	R	R-O
Unknown	O	O
	O	O
	O	O
	R	R-O
	R	R-O
	R	R-O
	R	R-O
	R	R-O
	R	R-O
	R	R-O
	R	R-O

*Genotype inferred after examination of 12 living offspring. Four were phenotype O.

(Cooper, 1966). Clearly, the classification of the foetuses into only two classes R/O and O should be considered a real result.

2. Dam to foetus transfer of blood-group substances

Transfer of R or O substance from the dam's circulation across the placenta to the foetal circulation which already contains O or R substance could result in the production of a mixed phenotype. If the frequency of the O phenotype is q^2, where q is the frequency of the r allele in the population, then with random mating the expected frequency of the mixed foetal blood groups can be derived from Table 46.

1) R dams carrying an O foetus are expected with a frequency q^2-q^3.

2) O dams carrying an R foetus are expected with a frequency of q^2-q^3.

Thus the expected total frequency of the R/O phenotype amongst foetuses is $2q^2-2q^3$ if the mixed phenotype is due to placental transfer alone. Similarly, the expected frequency of the unmixed phenotypes can be derived.

3) O dams carrying an O foetus are expected with a frequency of q^3.

4) R dams carrying an R foetus are expected with a frequency of $1-2q^2-q^3$.

TABLE 46

THE EXPECTED POPULATION FREQUENCIES OF OFFSPRING ARISING FROM VARIOUS MATING TYPES. q IS THE FREQUENCY OF r AND p = 1-q IS THE FREQUENCY OF R

Mating		Total Frequency of Mating in Population	Proportion Giving O Offspring	Proportion Giving R Offspring
F	M			
Rr	Rr	$4p^2q^2$	p^2q^2	$3p^2q^2$
Rr	rr	$2pq^3$	pq^3	pq^3
Total R dams with an O foetus			q^2-q^3	
rr	RR	p^2q^2	-	p^2q^2
rr	Rr	$2pq^3$	pq^3	pq^3
Total O dams with an R foetus				q^2-q^2
rr	Rr	$2pq^3$	pq^3	pq^3
rr	rr	p^4	q^4	-
Total O dams with an O foetus			q^3	
Rr	Rr	$4p^2q^2$	p^2q^2	$3p^2q^2$
Rr RR	RR Rr	) $4p^3q$)	-	$4p^3q$
Rr	rr	$2pq^3$	pq^3	pq^3
RR	RR	p^4	-	p^4
RR	rr	p^2q^3	-	p^2q^2
Total R dams with an R foetus				$1-2q^2+q^3$

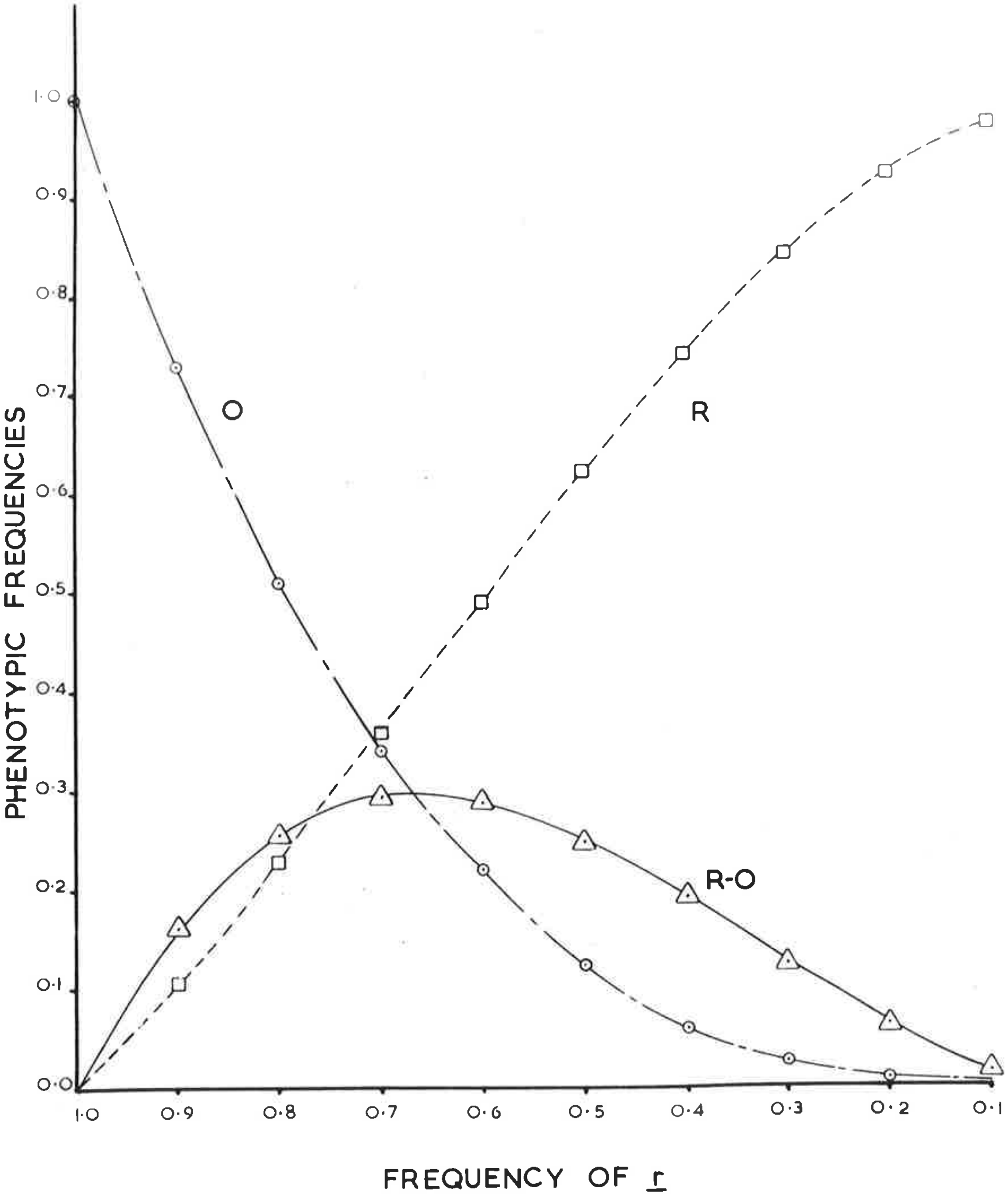
All of the derived expectations are presented in the form of a graph in Figure 28. From this it can be seen that the function $2q^2-2q^3$ has a maximum where $q = 0.67$. The observed frequency of the R/O phenotype is 0.87 which cannot be accounted for by any conditions of gene frequency with random mating. Also, the observed frequency of the O phenotype (0.13) and the R phenotype (0.0) are not in agreement with the mixing hypothesis. Different titres of both R and O substances in 3 of the 6 sets of twins in the data is further evidence against this hypothesis.

3. Different gene function in foetuses and adults

All adult sheep of genotype I-R- have O substance in their saliva and ii-- individuals do not (Rendel, 1957) indicating that the O substance is a product of the _I_ gene rather than the _r_ gene. It seems likely that in sheep foetuses, the enzyme which converts O substance into R substance in the liver of _R_- individuals has reduced activity. In this case one would expect to find O substance in all foetal sera except those of phenotype i. The observation that 9 foetuses had O substance alone is more difficult to explain on this basis since one might expect that in a sample of 69 sheep about 44 would be R (having both R and O substance) and 25 would be O when the frequency of the _r_ gene was 0.6 (a reasonable assumption, see Rendel _et al._, 1954; Rendel, 1957; Stansfield _et al._, 1964: Cooper, 1966). Either some _r/r_ foetuses also have R substance in their sera in which case the missing O individuals would be drawn

FIGURE 28.

Expected frequency of the R, O and R-O phenotypes of sheep foetuses for various values of q (= frequency of r) assuming that the frequency of r is the same for both sexes and that the R-O phenotype is due solely to the transfer of R and O substances from dam to foetus.


PHENOTYPIC FREQUENCIES
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0·0
O
R
R-O
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
FREQUENCY OF r

from the R/O category, or this deviation from expectation represents sampling variation. However, $\chi_1^2 = 16.1$ which has a probability of 0.001. Assuming a gene frequency of $q = 0.5$, the data agree more closely with the hypothesis; however, all reports suggest $q = 0.6 - 0.7$.

4. Different gene function and dam-foetus transfer

It is possible to account for the frequency of the observed phenotypes by invoking both the second and third hypotheses. Thus, all sheep foetuses have O substance in their serum because they carry the I gene (in this case). Some of these foetuses with O substance also have R substance because they also have the R gene (44) and the remainder (16) have the R substance because they received R substance from their dams *in utero*. There are thus 3 types of R/O foetus:

1) O foetuses carried in R ewes which may be expected with frequency $q^2 - q^3 = 0.14 = 8/69$ ($q = 0.6$)

2) R foetuses in O ewes which may be expected with frequency $q^2 - q^3 = 0.14 = 8/69$ ($q = 0.6$)

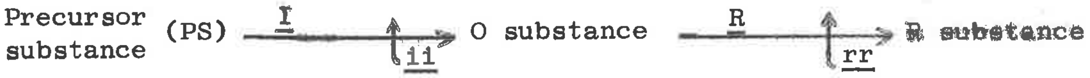
3) Foetuses of genotype I-R- regardless of dam's type may be expected with frequency $1 - q^2$ (0.64) or 44/69. This category would also include all those in class 2) above.

Thus, if $q = 0.6$ a proportion 0.78 or 54/69 would be expected to show the mixed phenotype and the remainder (0.22 or 15/69) would be expected to have the O antigen only. From Table 44 it can be seen that this is in agreement with the observed numbers of 60 and 9 ($\chi^2_1 = 3.07$). Further support to this idea is given by the observation that although 3 out of 6 sets of twins have members with different R and O titres, the remaining 3 have members with identical titres of one substance only. Lack of knowledge on the true frequency of r in the dams prevents further discussion along these lines. More information on the dam-foetus transfer and different gene function hypotheses to explain the mixed R-O phenotype of foetuses can be obtained from Table 45. As with the foetuses examined in Table 43, all have O substance in their serum whilst some also have R substance. If the mixed phenotype is entirely due to the presence of the I gene as it is in adults, it is surprising that "R" foetuses are only found in "R" dams especially where the sire is known to be heterozygous (R/r). It can be concluded that the phenotype of the foetus is probably not a reflection of its genotype but may be influenced by the phenotype of the dam possibly by trans-placental transfer of blood-group substance from dam to foetus.

The JOg typings of goat adults and foetuses (Table 43) show a basic difference from the sheep results, viz. about half of the foetuses have neither J nor Og substance in their serum and J adults do not have low titres of Og substance in their serum as do R sheep. The doe WPD which was type Og contained a foetus with neither J nor Og substance in its serum. There is thus no evidence for dam-foetus transfer in the limited goat data available.

The observation that the titres of R and O substances in foetal sheep serum are significantly correlated, especially at high titres, suggests that the model for gene action as proposed by Rendel (1957) may be modified slightly. Rendel's model may be summarized as follows:

In the liver

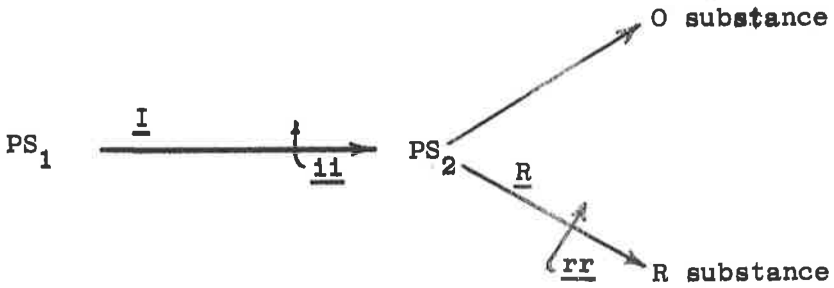


In salivary gland

The detection of both R and O substances in foetal sera would therefore suggest that in foetal liver:

where the enzyme produced by the I gene is of intermediate activity between the highly active form in adult liver, which results in the rapid conversion of O substance to R so that no O is detectable, and the relatively inactive form in salivary gland tissue.

This type of pathway however, would be expected to result in a negative correlation between the titre of R and O substance in RO foetuses since the titre of R substance would be positively correlated with the activity of the R enzyme and the titre of O substance negatively correlated with the activity of the R enzyme. A positive correlation could be explained by a branched pathway:



where the level of precursor substance 2 is determined by a gene which may or may not be the I gene.

The occurrence of a B phosphatase isozyme in the serum of foetuses is not related to either the age of foetuses, to the inhibition type or to the titre of R or O substance in the serum. This is a similar finding to that made on lambs by Rendel et al. (1964). However, a comparison of the mobility of the isozymes in adults, lambs and foetuses revealed that all had different mobility.

7 had an assoc and 10 [illegible], av O inhib titre for
B phos 3 7/45
no B phos 1 9/24

The failure to find an association between phosphatase type and the presence of O blood-group substance in the serum of lambs and foetuses is probably a reflection of the different isozymes present in these animals. Apparently the secretion of foetal and juvenile phosphatase into the serum is not affected by the R-O-i blood group type.

APPENDIX III

TABLES RELEVANT TO SECTION B

TABLE 47

TABLE OF MEANS OF TRANSFERRIN TYPES 1. JERSEY COWS

(a) Herd Level

Sample			Means of Transferrin Types						Difference between means						Analysis of Variance			
			A		AD		D		AD-A		D-A		D-AD		Mean Squares		⌿	Level of
Elements	Year	Number	No.	Mean	No.	Mean	No.	Mean	Mean	S.E.	Mean	S.E.	Mean	S.E.	Between	Within	V.R.	Significance
All cows	1963	420	145	369.1	205	387.0	70	378.7	17.9	7.9	9.6	11.0	-8.3	10.1	13,545	5,424	2.50	
	1964	486	174	372.5	238	387.3	74	389.0	14.8**	5.1	16.5*	6.9	1.7	7.0	12,998	2,632	4.94	*
First	1963	127	39	371.8	75	387.8	13	388.6	16.0	12.8	16.8	20.4	0.8	19.7	3,495	4,222	0.83	
Lactation	1964	103	39	367.9	54	388.6	10	407.1	20.7	10.6	39.2	15.7	18.5	18.1	8,163	2,473	3.30	
>2 Years	1963	293	106	368.1	130	386.5	57	376.4	18.4	10.1	8.3	12.9	-10.1	12.2	9,927	5,987	1.66	
old	1964	383	135	373.9	184	387.0	64	386.2	13.1	5.9	12.3	7.7	-0.8	7.6	7,304	2,681	2.72	
Mature	1963	89	32	406.1	44	384.2	13	431.1	-12.9	21.6	25.0	31.8	37.8	28.2	12,346	9,878	1.25	
Cows	1964	126	45	380.7	62	386.3	19	409.4	5.9	10.4	28.7	14.9	23.1	13.5	5,651	2,804	2.02	
<5 Years	1963	331	113	358.7	161	385.3	57	366.7	26.6**	8.0	8.1	10.5	-18.5	10.3	24,846	4,330	5.74	*
old	1964	360	129	369.7	176	387.7	55	382.0	18.0**	5.9	12.3	7.6	-5.8	8.1	12,186	2,553	4.77	

⌿ Variance ratio degrees of freedom = F^{2}_{n-3} where n = number of herds in the sample = 23 and 24 for 1963 and 1964

* $.01 < P < .05$

** $.001 < P < .01$

TABLE 48
TABLE OF MEANS OF TRANSFERRIN TYPES 1. JERSEY COWS
(b) Fat Percentages

Sample			Means of Transferrin Types						Difference between means						Analysis of Variance			
			A		AD		D		AD-A		D-A		D-AD		Mean Squares		⌿	Level of
Elements	Year	Number	No.	Mean	No.	Mean	No.	Mean	Mean	S.E.	Mean	S.E.	Mean	S.E.	Between	Within	V.R.	Significance
All cows	1963	420	145	5.228	205	5.335	70	5.449	0.11	0.06	0.22**	0.08	0.11	0.08	1.2085	0.3200	3.78	*
	1964	486	174	5.252	238	5.378	74	5.477	0.13*	0.05	0.23**	0.07	0.10	0.07	1.5306	0.2851	5.37	**
First	1963	127	39	5.351	75	5.331	13	5.654	-0.02	0.10	0.30	0.14	0.32	0.16	0.5885	0.2457	2.39	
Lactation	1964	103	39	5.269	54	5.285	10	5.290	0.02	0.08	0.02	0.12	0.01	0.14	0.0035	0.1518	0.03	
> 2 years	1963	293	106	5.183	130	5.337	57	5.402	0.15	0.08	0.22*	0.10	0.07	0.10	1.1035	0.3501	3.15	*
old	1964	383	135	5.247	184	5.405	64	5.506	0.16*	0.06	0.26**	0.09	0.10	0.08	1.7337	0.3198	5.42	**
Mature	1963	89	32	5.222	44	5.282	13	5.600	0.06	0.16	0.38	0.22	0.32	0.20	0.6879	0.4349	1.58	
Cows	1964	125	45	5.151	62	5.348	19	5.600	0.20	0.11	0.45**	0.16	0.25	0.14	1.4105	0.2973	4.74	**
< 5 Years	1963	331	113	5.230	161	5.349	57	5.414	0.12	0.07	0.18	0.08	0.07	0.09	0.7768	0.2913	2.67	
old	1964	360	129	5.287	176	5.389	55	5.435	0.10	0.06	0.15	0.08	0.05	0.09	0.5703	0.2803	2.03	

⌿ Variance Ratio degrees of freedom = F^{2}_{n-3} where n = number of cows in the sample

* $.01 < P < .05$

** $.001 < P < .01$

TABLE 49

TABLE OF MEANS OF TRANSFERRIN TYPES 1. JERSEY COWS

(c) Fat Yield

Sample			Means of Transferrin Types						Difference between means						Analysis of Variance			
			A		AD		D		AD-A		D-A		D-AD		Mean Squares		†	Level of
Elements	Year	Number	No.	Mean	No.	Mean	No.	Mean	Mean	S.E.	Mean	S.E.	Mean	S.E.	Between	Within	V.R.	Significance
All cows	1963	420	145	377.40	205	369.51	70	397.13	-7.89	11.70	19.73	14.77	27.62	15.96	19,994	11,833	1.68	
	1964	486	174	373.76	238	380.62	74	402.78	6.85	11.46	29.02	15.44	22.17	15.08	22,059	12,875	1.71	
First	1963	127	39	341.67	75	315.00	13	365.31	-26.67	16.15	23.64	21.75	50.31	26.17	19,020	6,584	2.89	
Lactation	1964	103	39	318.49	54	319.91	10	362.90	1.42	13.76	44.41	17.91	42.99	23.98	8,600	4,056	2.12	
>2 Years	1963	293	106	390.55	130	400.96	57	404.39	10.41	14.51	13.84	17.74	3.42	18.87	4,673	12,698	0.37	
old	1964	383	135	389.73	184	398.43	64	409.02	8.70	13.67	19.28	17.94	10.58	17.07	8,370	1,417	0.59	
Mature	1963	89	32	429.59	44	416.57	13	477.92	-13.03	28.76	48.33	45.66	61.36	37.54	18,898	15,930	1.19	
cows	1964	126	45	428.73	62	407.53	19	479.37	-21.20	25.24	50.64	33.70	71.83	31.28	37,798	15,481	2.44	
<5 Years	1963	331	113	362.62	161	356.65	57	378.70	-5.97	12.17	16.08	13.43	22.05	16.99	10,235	9,831	1.04	
old	1964	360	129	354.59	176	371.14	55	376.33	16.55	12.32	21.74	15.89	5.19	16.61	13,653	10,982	1.24	

† Variance Ratio degrees of freedom = F^{2}_{n-3} where n = number of cows in the sample

TABLE 50

TABLE OF MEANS OF TRANSFERRIN TYPES 1. JERSEY COWS

(d) Lactation Length

Sample			Means of Transferrin Types						Difference between means						Analysis of Variance			
			A		AD		D		AD-A		D-A		D-AD		Mean Squares		≁	Level of
Elements	Year	Number	No.	Mean	No.	Mean	No.	Mean	Mean	S.E.	Mean	S.E.	Mean	S.E.	Between	Within	V.R.	Significance
All cows	1963	420	145	9.717	205	9.595	70	9.686	-0.12	0.12	-0.03	0.12	0.09	0.18	0.6799	1.2803	0.53	
	1964	486	174	9.385	238	9.429	74	9.622	0.04	0.14	0.24	0.19	0.19	0.16	1.4978	1.7327	0.86	
First	1963	127	39	9.821	75	9.507	13	10.000	-0.31	0.27	0.18	0.17	0.49	0.45	2.1328	1.6814	1.27	
Lactation	1964	103	39	9.539	54	9.426	10	9.800	-0.11	0.23	0.26	0.29	0.37	0.40	0.6258	1.1250	0.56	
>2 Years	1963	293	106	9.679	130	9.646	57	9.614	-0.03	0.13	-0.07	0.15	-0.03	0.20	0.0826	1.1115	0.07	
old	1964	383	135	9.341	184	9.429	64	9.594	0.09	0.16	0.25	0.22	0.16	0.18	1.3921	1.9022	0.73	
Mature	1963	89	32	9.563	44	9.818	13	9.846	0.26	0.14	0.28	0.20	0.03	0.47	0.7077	0.3269	2.16	
Cows	1964	126	45	9.600	62	9.548	19	9.790	-0.05	0.19	0.19	0.25	0.24	0.22	0.4230	0.8399	0.50	
<5 Years	1963	331	113	9.761	161	9.534	57	9.649	-0.23	0.16	-0.11	0.14	0.12	0.23	1.7200	1.5292	1.12	
old	1964	360	129	9.310	176	9.386	55	9.564	0.08	0.17	0.25	0.24	0.18	0.21	1.2300	2.0416	0.61	

≁ Variance ratio degrees of freedom = F^{2}_{n-3} where n = number of cows in the sample.

TABLE 51

TABLE OF MEANS OF TRANSFERRIN TYPES 1. JERSEY COWS

(e) Milk Yield

Sample			Means of Transferrin Types						Difference between means						Analysis of Variance			
			A		AD		D		AD-A		D-A		D-AD		Mean Squares		⌿	Level of Significance
Elements	Year	Number	No.	Mean	No.	Mean	No.	Mean	Mean	S.E.	Mean	S.E.	Mean	S.E.	Between	Within	V.R.	
All cows	1963	420	145	721.61	205	692.43	70	724.76	-29.19	20.75	3.14	25.14	32.33	28.03	48,113	36,286	1.33	
	1964	486	174	712.33	238	707.00	74	736.43	- 5.34	20.45	24.10	26.87	29.45	26.43	24,608	40,034	0.62	
First Lactation	1963	127	39	637.96	75	592.19	13	646.85	-45.78	30.34	8.89	36.41	54.66	49.74	35,755	22,780	1.57	
	1964	103	39	605.49	54	606.33	10	687.90	0.85	25.44	82.41	34.35	81.57	43.81	30,306	13,897	2.18	
>2 Years old	1963	293	106	752.39	130	750.26	57	742.53	- 2.13	24.96	-9.87	29.62	-7.73	32.09	1,867	36,717	0.05	
	1964	383	135	743.20	184	736.54	64	744.01	- 6.66	24.34	0.82	30.92	7.48	29.95	2,305	43,784	0.05	
Mature Cows	1963	89	32	807.69	44	781.71	13	840.46	-25.98	41.20	32.77	67.70	88.75	51.80	18,945	32,730	0.58	
	1964	126	45	828.70	62	763.09	19	854.97	-65.61	43.82	26.27	54.28	91.86	55.23	89,470	45,539	1.96	
<5 Years old	1963	331	113	697.23	161	668.03	57	698.37	-29.21	23.11	1.13	25.11	30.34	31.55	36,216	34,610	1.05	
	1964	360	129	671.74	176	687.24	55	695.48	15.50	21.97	23.75	28.25	8.25	29.04	14,046	34,426	0.41	

⌿ Variance ratio degrees of freedom = F^{2}_{n-3} where n = number of cows in the sample

TABLE 52

TABLE OF MEANS OF TRANSFERRIN TYPES 1. JERSEY COWS

(f) Calving Interval

Sample			Means of Transferrin Types						Difference between means						Analysis of Variance			
			A		AD		D		AD-A		D-A		D-AD		Mean Squares		≠	Level of Significance
Elements	Year	Number	No.	Mean	No.	Mean	No.	Mean	Mean	S.E.	Mean	S.E.	Mean	S.E.	Between	Within	V.R.	
All Cows	1963	420	145	8.035	205	7.893	70	9.614	-0.14	0.71	1.88	0.90	1.72	0.89	81.50	40.89	1.99	
	1964	486	174	9.247	238	9.559	74	10.541	0.31	0.57	1.29	0.80	0.98	0.74	43.76	32.38	1.35	
First	1963	127	39	-	75	-	13	-	-	-	-	-	-	-	-	-	-	
Lactation	1964	103	39	-	54	-	10	-	-	-	-	-	-	-	-	-	-	
>2 Years	1963	293	106	10.991	130	12.169	57	11.807	1.18	0.57	0.82	0.73	0.73	-0.36	41.20	18.00	2.29	
old	1964	383	135	11.919	184	12.364	64	12.188	0.45	0.34	0.27	0.55	0.55	-0.18	7.73	9.67	0.80	
Mature	1963	89	32	12.375	44	13.023	13	12.923	0.65	0.43	0.55	0.43	0.43	-0.10	4.05	3.27	1.24	
cows	1964	126	45	12.978	62	12.968	19	13.421	-0.01	0.38	0.44	0.60	0.60	0.45	1.63	3.85	0.42	
<5 years	1963	331	113	6.805	161	6.471	57	8.860	-0.32	0.83	2.05	1.05	1.05	2.37	121.58	43.75	2.78	
old	1964	360	129	7.946	176	8.358	55	9.546	0.41	0.71	1.60	0.99	0.99	1.19	49.53	36.79	1.35	

≠ Variance ratio degrees of freedom = F^{2}_{n-3} where n = number of cows in the Sample

TABLE 53

TABLE OF MEANS OF TRANSFERRIN TYPES 1. JERSEY COWS

(g) Month of Calving

Sample			Means of Transferrin Types						Difference between means						Analysis of Variance			
			A		AD		D		AD-A		D-A		D-AD		Mean Squares		†	Level of
Elements	Year	Number	No.	Mean	No.	Mean	No.	Mean	Mean	S.E.	Mean	S.E.	Mean	S.E.	Between	Within	V.R.	Significance
All cows	1963	420	145	7.000	205	6.512	70	6.929	-0.49	0.34	-0.07	0.46	0.42	0.44	11.44	10.01	1.14	
	1964	486	174	6.615	238	6.265	74	7.000	-0.35	0.30	0.39	0.41	0.74	0.40	16.99	8.99	1.89	
First	1963	127	39	6.692	75	6.253	13	6.923	-0.44	0.61	0.23	0.99	0.67	0.97	4.05	9.75	0.42	
Lactation	1964	103	39	5.769	54	5.778	10	5.900	0.01	0.55	0.13	0.84	0.12	0.93	0.07	6.67	0.01	
> 2 Years	1963	293	106	7.113	130	6.662	57	6.930	-0.45	0.41	-0.18	0.53	0.27	0.51	6.05	10.18	0.60	
old	1964	383	135	6.859	184	6.408	64	7.172	-0.45	0.35	0.31	0.47	0.76	0.45	16.70	9.50	1.76	
Mature	1963	89	32	8.625	44	6.546	13	7.846	-2.08**	0.73	-0.78	1.06	1.30	1.07	41.06	10.47	3.92	*
cows	1964	126	45	7.622	62	6.855	19	7.263	-0.77	0.65	-0.36	0.88	0.41	0.92	7.74	11.32	0.68	
<5 Years	1963	331	113	6.540	161	6.503	57	6.719	-0.04	0.38	0.18	0.50	0.22	0.49	1.00	9.61	0.10	
old	1964	360	129	6.264	176	6.057	55	6.909	-0.21	0.33	0.65	0.46	0.85	0.44	15.22	8.01	1.90	

† Variance ratio degrees of freedom = F^{2}_{n-3} where n = number of cows in the sample

* $.01 < P < .05$

** $.001 < P < .01$

TABLE 54.

TABLE OF MEANS OF TRANSFERRIN TYPES 2. FRIESIAN COWS

(a) Herd Level

Sample			Means of Transferrin Types						Difference between means						Analysis of Variance			
			A		AD		D		AD-A		D-A		D-AD		Mean Squares		†	Level of Significance
Elements	Year	Number	No.	Mean	No.	Mean	No.	Mean	Mean	S.E.	Mean	S.E.	Mean	S.E.	Between	Within	V.R.	
All cows	1963	207	56	405.2	106	444.9	45	467.0	39.7**	12.1	61.8***	13.8	22.1	11.7	51,490	4,844	10.63	*
	1964	217	56	402.0	108	451.8	53	461.9	49.7***	11.6	59.9***	12.1	10.2	10.9	60,355	4,452	13.56	**
First Lactation	1963	66	18	413.9	30	443.4	18	482.6	29.5	21.7	68.7	22.4	39.2	21.2	21,442	5,000	4.29	
	1964	35	12	364.0	16	428.1	7	474.4	64.1*	25.2	110.4***	24.1	46.4	37.5	29,330	4,696	6.25	*
> 2 years old	1963	141	38	401.1	76	445.5	27	456.6	44.4**	14.7	55.5**	17.7	11.1	14.2	32,326	4,811	6.72	*
	1964	182	44	412.4	92	455.9	46	460.0	43.5**	12.9	47.6**	13.5	4.1	11.1	33,830	4,294	7.88	*
Mature cows	1963	58	16	398.1	33	452.1	9	481.2	54.0*	19.4	83.1**	24.1	29.1	21.4	24,011	3,619	6.64	*
	1964	73	17	407.1	41	469.7	15	470.7	62.6	22.2	63.6	28.3	0.96	19.8	25,807	4,949	5.21	
< 5 years old	1963	149	40	408.0	73	441.7	36	463.4	33.7	15.2	55.4	16.7	21.8	14.1	30,115	5,365	5.61	
	1964	144	39	399.8	67	440.8	38	458.5	40.9*	13.3	58.6**	14.4	17.7	12.8	35,662	4,133	8.63	*

† Variance Ratio degrees of freedom = F^{2}_{n-3} where n = number of herds in the sample = 8

* $.01 < P < .05$
** $.001 < P < .01$
*** $P < .001$

TABLE 55

TABLE OF MEANS OF TRANSFERRIN TYPES 2. FRIESIAN COWS

(b) Fat Percentage

Sample			Means of Transferrin Types						Difference between means						Analysis of Variance			
			A		AD		D		AD-A		D-A		D-AD		Mean Squares		†	Level of
Elements	Year	Number	No.	Mean	No.	Mean	No.	Mean	Mean	S.E.	Mean	S.E.	Mean	S.E.	Between	Within	V.R.	Significance
All cows	1963	207	56	3.929	106	3.761	45	3.649	-0.17*	0.08	-0.28***	0.08	-0.11	0.08	1.0229	0.1954	5.23	**
	1964	217	56	3.934	108	3.803	53	3.770	-0.13	0.08	-0.16	0.09	-0.03	0.09	0.4382	0.2406	1.82	
First	1963	66	18	3.894	30	3.843	18	3.694	-0.05	0.15	-0.20	0.14	-0.15	0.15	0.1996	0.2358	0.85	
Lactation	1964	35	12	3.775	16	3.975	7	3.971	0.20	0.18	0.20	0.22	-0.00	0.25	0.1560	0.2477	0.63	
>2 Years	1963	141	38	3.945	76	3.729	27	3.619	-0.22*	0.08	-0.33**	0.11	-0.11	0.10	0.9528	0.1785	5.34	**
old	1964	182	44	3.977	92	3.773	46	3.739	-0.20*	0.09	-0.24*	0.10	-0.03	0.09	0.7934	0.2363	3.36	**
Mature	1963	58	16	3.844	33	3.630	9	3.600	-0.21	0.10	-0.24	0.18	-0.03	0.13	0.2835	0.1278	2.22	
cows	1964	73	17	3.877	41	3.715	15	3.787	-0.16	0.11	-0.09	0.15	0.07	0.12	0.1610	0.1474	1.09	
<5 Years	1963	149	40	3.963	73	3.821	36	3.661	-0.14	0.10	-0.30**	0.09	-0.16	0.10	0.8606	0.2179	3.95	**
old	1964	144	39	3.959	67	3.857	38	3.763	-0.10	0.11	-0.20	0.11	-0.09	0.11	0.3696	0.2877	1.29	

† Variance ratio degrees of freedom = F^{2}_{n-3} where n = number of cows in the sample

* $.01 < P < .05$

** $.001 < P < .01$

*** $P < .001$

TABLE 56

TABLE OF MEANS OF TRANSFERRIN TYPES 2. FRIESIAN COWS

(c) Fat Yield

Sample			Means of Transferrin Types						Difference between means						Analysis of Variance			
			A		AD		D		AD-A		D-A		D-AD		Mean Squares		≠	Level of
Elements	Year	Number	No.	Mean	No.	Mean	No.	Mean	Mean	S.E.	Mean	S.E.	Mean	S.E.	Between	Within	V.R.	Significance
All cows	1963	207	56	436.46	106	451.78	45	430.09	15.32	20.17	-6.38	22.10	-21.69	20.51	9,035	13,656	0.66	
	1964	217	56	390.48	108	436.42	53	426.19	45.94	20.48	35.71	23.97	-10.23	20.60	39,502	15,371	2.57	
First	1963	66	18	376.11	30	397.30	18	396.94	21.19	24.55	20.83	23.10	- 0.36	24.86	2,903	6,311	0.46	
Lactation	1964	35	12	303.92	16	349.00	7	363.57	45.08	33.21	59.66	37.23	14.57	46.30	10,185	8,124	1.25	
> 2 Years	1963	141	38	465.05	76	473.29	27	452.19	8.24	25.55	-12.86	30.30	-21.10	27.34	4,538	15,467	0.29	
old	1964	182	44	414.09	92	451.62	46	435.72	37.52	22.67	21.63	26.83	-15.90	21.92	21,203	15,306	1.39	
Mature	1963	58	16	481.75	33	518.18	9	483.67	36.43	36.95	1.92	50.13	-34.52	41.28	9,096	13,695	0.66	
cows	1964	73	17	412.41	41	497.56	15	466.80	38.15	36.09	54.39	52.96	-30.76	39.27	43,765	17,585	2.49	
< 5 Years	1963	149	40	418.35	73	421.76	36	416.69	3.42	22.38	-1.66	23.81	- 5.07	22.06	355	11,939	0.03	
old	1964	144	39	380.92	67	399.00	38	410.16	18.08	23.11	29.24	25.77	11.16	21.95	8,463	12,518	0.66	

≠ Variance ratio degrees of freedom = F^{2}_{n-3} where n = number of cows in the sample

TABLE 57

TABLE OF MEANS OF TRANSFERRIN TYPES 2. FRIESIAN COWS

(d) Lactation Length

Sample			Means of Transferrin Types						Difference between means						Analysis of Variance			
			A		AD		D		AD–A		D–A		D–AD		Mean Squares		⌿	Level of
Elements	Year	Number	No.	Mean	No.	Mean	No.	Mean	Mean	S.E.	Mean	S.E.	Mean	S.E.	Between	Within	V.R.	Significance
All cows	1963	207	56	9.923	106	9.877	45	9.978	−0.05	0.06	0.05	0.04	0.10	0.07	0.1685	0.1181	1.43	
	1964	217	56	9.768	108	9.704	53	9.566	−0.06	0.14	−0.20	0.24	−0.14	0.21	0.5859	1.2687	0.46	
First	1963	66	18	10.000	30	9.967	18	10.000	−0.03	0.04	0.00	-	0.03	0.04	0.0091	0.0153	0.59	
Lactation	1964	35	12	9.917	16	9.813	7	9.857	−0.10	0.7	−0.06	0.15	0.05	0.23	0.0372	0.1941	0.19	
> 2 Years	1963	141	38	9.895	76	9.842	27	9.963	−0.05	0.09	0.07	1.01	0.12	0.10	0.1516	0.1641	0.92	
old	1964	182	44	9.727	92	9.685	46	9.522	−0.04	0.17	−0.21	0.29	−0.16	0.24	0.5640	1.4752	0.38	
Mature	1963	58	16	9.813	33	9.788	9	10.000	−0.03	0.15	0.19	0.14	0.21	0.18	0.1616	0.2173	0.74	
cows	1964	73	17	9.765	41	9.781	15	9.133	0.02	0.17	−0.63	0.60	−0.65	0.40	2.4616	1.4259	1.73	
< 5 Years	1963	149	40	9.975	73	9.918	36	9.972	−0.06	0.06	−0.00	0.04	0.05	0.06	0.0582	0.0785	0.74	
old	1964	144	39	9.769	67	9.657	38	9.737	−0.11	0.20	−0.03	0.24	0.08	0.24	0.1770	1.1872	0.15	

⌿ Variance ratio degrees of freedom = F^{2}_{n-3} where n = number of cows in the sample

TABLE 58

TABLE OF MEANS OF TRANSFERRIN TYPES 2. FRIESIAN COWS
(e) Milk Yield

Sample			Means of Transferrin Types						Differences between means						Analysis of Variance			
			A		AD		D		AD-A		D-A		D-AD		Mean Squares			
Elements	Year	Number	No.	Mean	No.	Mean	No.	Mean	Mean	S.E.	Mean	S.E.	Mean	S.E.	Between	Within	† V.R.	Level of Significance
All cows	1963	207	56	1113.47	106	1199.01	45	1186.10	85.53	52.23	72.62	52.03	- 12.91	53.84	138.918	89,207	1.56	
	1964	217	56	988.96	108	1160.31	53	1133.51	171.35***	51.78	144.55**	56.51	- 26.81	53.14	561,520	96,465	5.82	**
First	1963	66	18	971.89	30	1026.45	18	1066.17	54.56	57.79	94.28	48.96	39.72	53.14	40,448	31,135	1.30	
Lactation	1964	35	12	806.25	16	878.16	7	920.57	71.91	78.37	114.32	92.35	42.42	106.04	32,744	45,093	0.73	
$>$ 2 Years	1963	141	38	1180.54	76	1267.19	27	1266.06	86.58	66.34	85.52	71.34	7.06	72.26	103,382	101,650	1.02	
old	1964	182	44	1038.80	92	1209.39	46	1165.91	170.59**	56.92	127.12*	62.73	- 43.47	55.73	435,453	94,020	4.63	*
Mature	1963	58	16	1248.16	33	1405.89	9	1340.67	157.74	100.62	92.51	112.54	- 65.23	116.13	134.769	96,543	1.40	
cows	1964	73	17	1061.50	41	1347.28	15	1239.23	285.78**	91.93	177.73	131.94	-108.05	100.16	494,241	112,849	4.38	*
$<$ 5 Years	1963	149	40	1059.60	73	1105.48	36	1147.46	45.88	54.14	87.86	56.05	41.98	53.96	73,466	69,604	1.06	
old	1964	144	39	957.35	67	1045.70	38	1091.78	88.56	55.49	135.43	57.48	45.87	54.68	182,749	71,430	2.56	

† Variance ratio degrees of freedom = F^{2}_{n-3} where n = number of cows in the sample.

* $.01 < P < .05$

** $.001 < P < .01$

*** $P < .001$

TABLE 59

TABLE OF MEANS OF TRANSFERRIN TYPES 2. FRIESIAN COWS

(f) Calving Interval

Sample			Means of Transferrin Types						Difference between means						Analysis of Variance			
			A		AD		D		AD-A		D-A		D-AD		Mean Squares		†	Level of
Elements	Year	Number	No.	Mean	No.	Mean	No.	Mean	Mean	S.E.	Mean	S.E.	Mean	S.E.	Between	Within	V.R.	Significance
All cows	1963	207	56	8.464	106	8.887	45	7.289	0.42	1.08	-1.18	1.33	-1.60	1.18	40.387	43.479	0.93	
	1964	217	56	10.179	108	10.722	53	10.811	0.54	0.92	0.63	1.04	0.09	0.87	6.961	29.178	0.24	
First	1963	66	18	-	30	-	18	-	-	-	-	-	-	-	-	-	-	
Lactation	1964	35	12	-	16	-	7	-	-	-	-	-	-	-	-	-	-	
> 2 Years	1963	141	38	12.474	76	12.395	27	12.148	-0.08	0.76	-0.33	0.95	-0.25	0.90	0.892	15.007	0.06	
old	1964	182	44	12.705	92	12.587	46	12.457	-0.12	0.61	-0.25	0.62	-0.13	0.53	0.694	9.614	0.07	
Mature	1963	58	16	13.125	33	13.727	9	12.667	0.60	0.96	-0.46	0.61	-1.06	1.25	4.792	8.769	0.55	
cows	1964	73	17	13.647	41	13.049	15	12.733	-0.60	0.59	-0.91	0.81	-0.32	0.48	3.586	3.758	0.96	
< 5 Years	1963	149	40	6.600	73	6.699	36	5.944	0.10	1.29	-0.66	1.59	-0.75	1.34	7.188	44.198	0.16	
old	1964	144	39	8.667	67	9.299	38	10.053	0.63	1.28	1.39	1.34	0.75	1.22	18.534	37.252	0.50	

† Variance ratio degrees of freedom = F^{2}_{n-3} where n = number of cows in the sample

TABLE 60

TABLE OF MEANS OF TRANSFERRIN TYPES 2. FRIESIAN COWS

(g) Month of Calving

Sample			Means of Transferrin Types						Difference between means						Analysis of Variance			
			A		AD		D		AD-A		D-A		D-AD		Mean Squares		⌿	Level of
Elements	Year	Number	No.	Mean	No.	Mean	No.	Mean	Mean	S.E.	Mean	S.E.	Mean	S.E.	Between	Within	V.R.	Significance
All cows	1963	207	56	6.732	106	7.113	45	7.133	0.38	0.54	0.40	0.64	0.02	0.58	3.066	10.602	0.29	
	1964	217	56	6.857	108	6.852	53	6.585	-0.01	0.58	-0.27	0.68	-0.27	0.58	1.447	12.249	0.12	
First	1963	66	18	8.333	30	6.533	18	7.222	-1.80	1.00	-1.11	1.09	0.69	1.07	18.226	11.755	1.55	
Lactation	1964	35	12	5.250	16	4.750	7	3.429	-0.50	1.02	-1.82	1.23	-1.32	1.29	7.461	7.343	1.02	
> 2 Years	1963	141	38	5.974	76	7.342	27	7.074	1.37	0.63	1.10	0.76	-0.27	0.70	24.106	9.760	2.48	
old	1964	182	44	7.296	92	7.217	46	7.065	-0.08	0.65	-0.23	0.75	-0.15	0.61	0.632	12.199	0.05	
Mature	1963	58	16	4.938	33	7.546	9	8.444	2.61**	0.95	3.51**	1.22	0.90	1.11	48.295	9.115	5.30	**
cows	1964	73	17	7.177	41	8.024	15	7.667	0.85	0.96	0.49	1.20	-0.36	0.87	4.391	10.068	0.44	
< 5 Years	1963	149	40	7.450	73	6.918	36	6.806	-0.53	0.64	-0.64	0.72	-0.11	0.69	4.893	10.692	0.46	
old	1964	144	39	6.718	67	6.134	38	6.158	-0.58	0.11	-0.56	0.83	0.02	0.72	4.709	12.757	0.37	

⌿ Variance ratio degrees of freedom = F^{2}_{n-3} where n = number of cows in the sample.

** $.001 < P < .05$

TABLE 61

MEANS AND STANDARD ERRORS FOR EACH PROGENY GROUP. 420 JERSEY COWS, 1963 CALVING

Progeny Group	Number Cows	Fat %		Fat Yield		Milk Yield		Lactation Length		Calving Interval		Month Calving		Age	
		Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1	13	5.90	0.19	473.92	18.43	804.12	25.12	9.69	0.13	9.92	1.55	7.15	0.75	4.46	0.57
2	29	4.90	0.10	313.38	18.97	633.05	38.49	9.31	0.38	8.21	1.36	8.03	0.43	2.88	0.18
3	19	4.44	0.10	259.84	17.68	577.58	42.05	9.05	0.44	13.47	0.97	7.42	0.55	0.55	0.32
4	8	4.49	0.17	314.38	28.66	702.38	57.75	9.50	0.31	10.13	2.12	9.00	0.56	3.38	0.33
5	16	5.71	0.07	303.00	10.45	532.31	21.11	9.63	0.22	4.06	1.54	7.19	1.07	2.75	0.19
6	5	5.62	0.09	272.40	7.27	487.20	18.71	10.00	0.00	0.00	0.00	6.80	0.45	2.00	0.00
7	7	5.89	0.19	406.86	27.98	686.50	31.80	10.00	0.00	3.43	2.07	4.71	1.59	2.14	0.13
8	11	5.45	0.14	477.18	21.65	879.41	43.19	10.00	0.00	13.36	0.57	6.27	1.42	5.32	0.59
9	11	5.53	0.10	452.09	16.51	817.91	29.05	10.00	0.00	10.18	1.48	7.45	1.27	3.68	0.34
10	5	5.98	0.03	266.60	72.12	443.70	117.99	7.40	1.54	2.20	2.01	6.40	1.99	2.20	0.18
11	15	5.53	0.08	431.73	27.16	793.93	53.50	10.00	0.00	4.80	1.53	6.40	1.05	2.27	0.10
12	12	5.37	0.07	360.67	21.55	671.75	40.97	9.67	0.25	5.92	1.73	4.08	0.45	2.58	0.31
13	12	5.87	0.10	437.75	22.83	748.46	39.15	9.83	0.11	8.92	1.50	4.50	0.42	3.42	0.29
14	12	4.72	0.11	303.33	15.70	639.63	34.53	9.67	0.18	7.08	1.74	6.58	0.79	2.58	0.18
15	9	5.18	0.15	348.78	25.12	674.00	48.07	9.33	0.27	12.44	0.28	7.11	1.20	4.22	0.29
16	10	6.23	0.20	639.00	32.68	1027.15	43.52	10.00	0.00	12.70	0.51	6.50	1.06	6.70	0.25
17	10	5.43	0.17	447.50	31.80	823.65	51.80	9.60	0.21	12.10	0.33	7.40	0.92	7.50	0.46
18	13	5.28	0.09	336.38	17.76	638.65	36.16	9.54	0.18	6.85	1.80	5.92	0.70	2.65	0.20
19	4	5.37	0.17	306.50	32.01	567.00	51.53	9.25	0.22	0.00	0.00	7.50	0.44	2.00	0.00
20	12	5.16	0.10	357.25	11.11	693.00	18.53	9.50	0.22	12.25	0.13	7.67	0.34	5.63	0.28
21	14	5.40	0.12	363.07	17.56	674.68	29.53	9.93	0.07	8.50	1.72	7.00	1.00	3.64	0.47
22	12	5.57	0.09	354.42	20.75	635.13	33.79	9.83	0.16	7.92	1.95	5.92	1.18	2.92	0.24
23	11	5.39	0.11	407.36	28.69	755.18	48.69	9.73	0.19	10.36	2.04	7.27	0.95	3.27	0.36
24	19	5.35	0.07	376.11	14.11	701.84	25.13	9.68	0.11	2.95	1.38	5.53	0.27	2.74	0.15
25	10	5.40	0.14	449.00	28.59	841.65	62.56	10.00	0.00	9.60	1.54	10.20	0.81	4.60	0.50
26	10	5.16	0.18	366.50	52.89	705.00	92.87	8.90	0.85	13.40	1.06	8.00	1.01	6.30	0.38
27	5	5.52	0.16	354.60	24.58	647.40	57.16	10.00	0.00	0.00	0.00	7.20	1.27	2.00	0.00
28	14	4.96	0.11	484.14	17.87	986.96	44.92	9.79	0.11	9.00	1.57	4.21	0.40	3.11	0.18
29	11	5.40	0.08	342.91	15.68	634.09	28.99	9.64	0.27	11.27	1.68	7.45	1.10	3.77	0.23
30	7	5.39	0.15	367.43	27.88	674.79	33.28	10.00	0.00	2.14	2.00	3.43	1.17	2.64	0.24
31	23	5.05	0.07	334.43	8.80	661.76	15.07	10.00	0.00	7.22	1.22	6.57	0.51	2.67	0.15
32	7	6.27	0.11	373.29	45.12	591.57	70.32	8.86	1.07	0.00	0.00	5.43	0.40	2.00	0.00
33	13	5.10	0.11	443.00	28.13	872.00	55.75	10.00	0.00	10.85	1.45	7.77	0.61	3.42	0.48
34	10	5.27	0.14	419.30	30.18	798.60	56.63	9.60	0.29	9.60	1.53	6.60	0.92	3.25	0.30
35	21	5.26	0.07	338.14	13.01	645.83	25.92	9.81	0.09	8.57	1.34	7.52	0.48	2.74	0.15
Total	420														

TABLE 62

MEANS AND STANDARD ERRORS FOR EACH PROGENY GROUP. 486 JERSEY COWS, 1964 CALVING

Progeny Group	Number Cows	Fat %		Fat Yield		Milk Yield		Lactation Length		Calving Interval		Month Calving		Age	
		Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1	13	5.94	0.19	455.08	20.54	769.04	32.37	9.77	0.12	12.38	0.30	6.23	0.79	5.35	0.53
2	25	5.14	0.07	336.00	16.63	652.26	30.58	9.60	0.20	12.28	1.07	8.12	0.50	3.76	0.24
3	20	5.00	0.12	286.70	18.01	576.63	36.72	9.10	0.27	13.45	0.84	8.70	0.68	5.20	0.32
4	8	4.76	0.16	253.50	29.18	530.75	56.12	8.25	0.66	12.13	1.71	10.00	0.85	4.00	0.30
5	17	5.93	0.12	300.06	23.63	513.94	41.79	8.65	0.61	7.06	1.44	6.65	0.96	3.35	0.22
6	12	5.55	0.10	305.08	24.70	547.21	41.49	9.08	0.66	5.17	1.80	5.00	0.97	2.38	0.11
7	8	5.91	0.16	445.63	34.46	745.13	39.03	10.00	0.00	8.38	2.34	4.00	1.14	2.81	0.25
8	9	5.61	0.18	525.89	36.18	934.22	57.35	10.00	0.00	13.22	0.44	7.22	1.16	5.44	0.31
9	11	5.61	0.11	493.64	21.18	881.45	36.73	9.91	0.09	13.09	0.30	6.55	1.41	4.59	0.32
10	4	5.67	0.12	458.75	17.94	810.25	33.72	10.00	0.00	0.00	0.00	4.50	0.78	2.00	0.00
11	13	5.74	0.14	510.62	28.71	900.31	60.73	9.85	0.10	10.46	1.27	5.15	0.85	3.12	0.17
12	12	5.38	0.08	350.33	36.65	652.25	67.60	8.00	0.70	12.17	0.31	4.17	0.46	3.50	0.33
13	14	5.73	0.12	365.93	34.40	636.14	56.26	8.29	0.57	9.93	1.14	4.57	0.44	3.89	0.30
14	13	4.67	0.15	314.92	20.87	674.15	39.63	9.38	0.28	11.15	0.93	6.92	0.68	3.35	0.24
15	9	4.79	0.18	358.56	35.05	754.39	74.09	9.56	0.28	12.67	0.57	5.22	1.08	5.28	0.34
16	9	5.81	0.17	638.33	42.40	1098.00	62.99	10.00	0.00	13.22	0.63	7.11	1.37	7.33	0.22
17	6	5.43	0.24	479.00	37.61	881.17	61.55	9.67	0.31	12.67	0.66	6.50	0.78	7.67	0.52
18	13	5.35	0.07	397.46	25.08	746.31	50.30	9.54	0.18	9.08	1.43	5.54	0.67	3.04	0.26
19	12	5.12	0.09	330.92	22.13	647.08	39.96	9.42	0.19	3.83	1.57	6.08	0.51	2.17	0.07
20	12	5.12	0.11	356.83	19.00	693.50	29.08	9.58	0.22	12.00	0.12	7.67	0.34	6.58	0.28
21	16	5.29	0.10	364.13	14.73	689.25	28.80	9.94	0.06	10.06	1.50	7.19	0.84	3.84	0.43
22	9	5.56	0.06	352.00	18.29	635.67	37.14	9.78	0.21	12.11	1.45	6.67	1.06	3.61	0.31
23	12	5.29	0.10	440.75	37.49	828.46	67.79	9.33	0.64	11.92	1.29	6.58	0.94	4.17	0.39
24	31	5.41	0.07	391.39	17.72	718.40	32.18	9.16	0.18	6.65	1.02	5.45	0.24	2.95	0.18
25	10	5.58	0.11	398.00	34.72	718.70	69.97	9.90	0.10	10.00	1.64	7.40	1.37	4.80	0.66
26	7	5.30	0.22	396.57	32.02	757.07	57.08	9.57	0.19	13.86	1.23	6.86	1.55	7.29	0.53
27	11	5.38	0.09	334.55	28.97	624.77	53.51	8.73	0.75	5.45	1.81	7.36	0.81	2.32	0.12
28	18	5.00	0.10	421.89	21.47	856.08	53.14	9.50	0.21	8.89	1.14	4.00	0.31	3.58	0.27
29	10	5.53	0.09	361.60	24.12	655.15	44.36	9.70	0.29	13.00	0.45	7.70	1.12	4.65	0.16
30	8	5.69	0.19	387.13	30.15	677.94	43.10	9.63	0.25	7.38	2.67	6.63	0.89	3.06	0.26
31	31	5.00	0.07	349.45	14.82	696.29	26.67	9.65	0.13	9.58	1.07	6.77	0.41	3.26	0.19
32	11	6.08	0.12	463.18	22.16	758.95	27.97	10.00	0.00	7.00	1.98	6.18	0.62	2.50	0.14
33	17	5.05	0.09	417.59	24.56	832.50	48.56	9.24	0.28	9.35	1.27	6.94	0.64	3.79	0.45
34	12	5.30	0.10	427.83	20.15	809.00	36.26	10.00	0.00	9.25	1.56	6.08	0.76	3.63	0.39
35	31	5.26	0.09	341.84	14.33	652.71	27.90	9.55	0.26	8.58	1.09	7.55	0.29	3.18	0.19
36	12	5.17	0.07	315.25	12.12	611.13	24.78	9.92	0.08	0.00	0.00	6.08	0.81	2.04	0.04
Total	486														

TABLE 63

MEANS AND STANDARD ERRORS FOR EACH PROGENY GROUP. 207 FRIESIAN COWS 1963 CALVING

Progeny Group	Number Cows	Fat %		Fat Yield		Milk Yield		Lactation Length		Calving Interval		Month Calving		Age	
		Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1	9	3.81	0.08	501.89	24.33	1332.00	76.10	10.00	0.00	12.00	1.43	5.33	0.91	4.39	0.34
2	10	3.72	0.11	611.50	32.67	1580.25	101.91	10.00	0.00	13.20	0.31	7.80	1.09	6.20	0.54
3	14	4.60	0.06	546.07	26.42	1194.86	59.03	10.00	0.00	3.43	1.45	5.14	0.74	2.50	0.18
4	6	3.65	0.07	355.33	21.90	972.50	46.92	10.00	0.00	4.00	2.34	10.50	0.85	2.42	0.25
5	21	3.78	0.08	465.71	17.59	1237.64	46.38	9.86	0.10	11.43	1.12	11.43	1.12	4.02	0.29
6	20	3.63	0.09	390.20	14.86	1069.20	44.06	9.90	0.10	1.65	0.88	7.70	0.89	2.07	0.04
7	8	3.12	0.15	465.75	34.83	1285.13	57.50	10.00	0.00	11.75	0.35	11.75	0.35	4.00	0.35
8	8	4.01	0.20	419.00	51.82	1039.13	97.54	9.85	0.12	1.50	1.41	9.00	0.36	2.25	0.15
9	33	3.51	0.07	480.91	18.17	1371.48	46.63	9.94	0.04	11.73	1.06	7.30	0.62	5.06	0.38
10	12	3.85	0.09	391.25	20.50	1011.00	43.92	10.00	4.00	4.00	1.64	6.33	0.71	2.38	0.13
11	13	3.70	0.08	450.92	20.32	1222.00	47.96	10.00	0.00	8.85	1.66	7.15	0.65	3.62	0.41
12	22	3.80	0.09	357.18	17.47	933.95	49.10	9.73	0.12	13.14	0.52	6.41	0.67	4.61	0.31
13	13	3.97	0.07	305.31	20.70	770.77	49.08	9.85	0.15	6.38	1.93	7.46	0.86	2.85	0.26
14	18	3.67	0.10	464.44	27.12	1258.75	63.00	9.89	0.07	7.28	1.58	6.11	0.78	3.11	0.26
Total	207														

TABLE 64

MEANS AND STANDARD ERRORS FOR EACH PROGENY GROUP. 217 FRIESIAN COWS 1964 CALVING

Progeny Group	Number Cows	Fat %		Fat Yield		Milk Yield		Lactation Length		Calving Interval		Month Calving		Age	
		Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1	9	3.96	0.06	552.44	23.14	1402.90	71.43	10.00	0.00	13.11	0.11	6.33	0.99	5.56	0.37
2	7	3.76	0.14	594.00	27.75	1593.43	82.11	10.00	0.00	14.00	0.50	8.57	0.89	7.00	0.65
3	19	4.75	0.10	522.26	30.34	1119.66	74.05	9.53	0.34	9.11	1.52	5.05	0.78	3.05	0.23
4	11	3.76	0.11	434.45	26.46	1150.23	57.80	10.00	0.00	12.82	0.17	5.55	1.23	3.36	0.16
5	19	3.58	0.13	412.89	26.36	1154.21	69.82	9.37	0.30	12.74	0.41	6.68	0.68	5.10	0.29
6	17	3.65	0.11	420.71	20.64	1163.82	53.41	9.76	0.23	12.12	1.13	7.82	1.04	2.91	0.10
7	8	3.94	0.14	398.88	62.85	1022.94	165.27	7.75	1.29	13.13	0.58	8.50	0.74	5.25	0.35
8	9	3.89	0.13	332.00	24.88	856.83	61.67	9.00	0.39	8.22	2.06	5.22	1.30	2.72	0.25
9	28	3.61	0.07	476.68	20.25	1319.21	48.90	9.93	0.05	12.54	0.55	7.50	0.64	5.80	0.42
10	12	3.95	0.05	384.08	20.23	967.88	47.10	9.92	0.08	12.17	1.11	7.33	0.72	3.50	0.16
11	14	3.76	0.07	417.86	16.08	1116.29	36.78	9.93	0.07	12.71	1.15	8.57	0.76	4.61	0.43
12	10	3.59	0.10	304.00	24.74	859.25	81.70	9.50	0.21	13.10	0.78	6.70	1.39	5.40	0.64
13	19	3.91	0.09	275.79	14.11	705.84	34.04	9.63	0.19	6.89	1.52	6.68	0.83	3.08	0.28
14	21	3.68	0.09	460.00	21.91	1249.17	47.90	10.00	0.00	9.86	1.06	5.81	0.69	3.71	0.27
15	14	3.71	0.06	354.29	13.88	931.39	40.01	10.00	0.00	0.00	0.00	6.43	0.58	2.11	0.06
Total	217														

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PUBLISHED PAPERS

1. Specific milk proteins associated with resumption of development by the quiescent blastocyst of the lactating red kangaroo.

2. A specific protein difference in the milk from two mammary glands of a red knagaroo.

3. Serum transferrins of twin sheep-goat hybrids.

4. The haemoglobins of a sheep-goat hybrid foetus and those of the parent species.

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