An Examination of Genetic Variation in Plains Bison (*Bison bison bison*), Wood Bison (*B.b. athabascae*) and their Hybrids

A report submitted by P.W. Neumann in fulfillment of contract CWS7172-040

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Introduction

Plains bison (*Bison bison bison*) and wood bison, (*B.b. athabascae*) originated from *Bison occidentalis* which existed at the time of the Wisconsin glaciation (Novakowski, unpublished). The known hybrids of these subspecies originated in modern times as a result of a transplant of plains bison from Wainwright National Park, Alberta into Wood Buffalo National Park where bison were indigenous (Flerov and Zablotski, 1961).

At present, the classification of subspecies of bison is based on rather nebulus morphological (size, pelage, sexual dimorphism, etc.) and ethological characteristics. A somewhat more quantitative method entails morphometric analyses of bone structure (Novakowski, unpublished). These latter methods are usually on dead animals. The present population of wood bison, however, is about seventy-five (Novakowski, 1970) thus necessitating the invocation of classification techniques which can be performed on live animals or their tissues.

Reasons for wishing to manage bison (and in particular the maintenance of pure strains) are enumerated

by Fuller (1966) as: (i) its aesthetic and historical values, (ii) its value as a source of human food, (iii) its value as potential game. As the wood bison are endangered mammals it becomes increasingly important to have them 'mapped' genetically so that the pure strains can be maintained and perpetuated.

In recent years, new techniques, utilizing zone electroporesis followed by histrochemical staining methods to demonstrate zones of enzyme activity directly on the electrophoretic medium, have provided a method for analysis of genetic variation. Any mutation in a structural gene results in the substitution, deletion or addition of one or more amino acids. Such a change will, in some cases, result in a change in net electrophoretic charge on the enzyme of which such a polypeptide is a constituent (Hubby and Lewontin, 1966). MacCluer (cited in Shaw, 1965) has calculated that a single substitution in the nucleotide will produce a change of net charge in 27.56% of the enzymes. MacCluer's calculation is theoretical and requires numerous assumptions. The actual percentage that is detectable is unknown. Theoretically however, three-fourths of all mutants will not be electrophoretically detectable.

In cattle, hemoglobin examined by electrophoresis is found as one band, HbA (Rendel, 1967). Other Hb types occur in Southern European cattle and Asian cattle, the most common being HbB (Efremov and Braend, 1965).

The European bison (Bison bonasus) has an identical two band pattern, the slower migrates at the same rate as HbA of cattle, the faster at the same rate as Hb[illegible] (Efremov and Braend, op. cit.). In four bison examined, all had the same hemoglobin phenotype (Braend and Gasparski, 1967). Braend and Stormont (1963) also found no polymorphism in Hb or transferrins of 113 American buffalo. The transferrin pattern in bison was found identical to the AA pattern in cattle. The transferrins (Tf) in both B. bonasus and B. bison appear as a three band pattern with a very faint fourth band in front (Braend and Gasparski, 1967). No Tf polymorphism was exhibited in 14 European bison examined by Braend and Gasparski (op. cit.).

Using the starch gel method of Kristjansson (1963), the bison transferrin pattern can be distinguished from the AA pattern of cattle. This new technique elicts four principle bands, none of which is precisely synchronous with any of the principle bands in cattle (Stormont, 1964).

It appears then that the genera Bos and Bison are distinguishable on the basis of the transferrins as well as their hemoglobins. Ten or eleven phenotypes of up to eight components are now detectable in cattle transferrins (Quinteros and Miller, 1968). Transferrin patterns in Atlantic salmon have been used to taxonomically split the species (Salmo salar) into two subspecific taxa (Payne, Child and Forrest, 1971). These authors found two phenotypes exclusive to European salmon and five phenotypes restricted to North American Atlantic salmon.

Naik and Anderson (1970) studied the glucose-6-phosphate dehydrogenase (G-6PD) and 6-phosphogluconate dehydrogenase (G-PGD) loci of red cells in American bison (B. bison). None of the 86 bison sampled indicated detectable variation for G-6PD. Three phenotypes, however, were revealed at the 6-PGD locus which were presumed to be determined by codominant alletic genes Pd^A and Pd^B.

Three zones of carbonic anhydrase activity (CA^1, CA^2 and CA^3) were observed in American bison (Sartore et. al., 1969). These occurred in five or six expected phenotypes based on control vested in three co-dominant alleles CA^1, CA^2 and CA^3.

Observed numbers in each case agreed with expected numbers assuming Hardy-Weinberg equilibrium. Bison zones CA^1 and CA^2 migrated ahead of CA-F of cattle whereas CA^3 appeared to migrate at about the same rate as CA-S of cattle.

As the genetics of transferrins in cattle has been extensively studied (Jamieson, 1965; Kristjansson and Hickman, 1965 and Ashton, 1965), the closely related genus, Bison is investigated in the present study. The revelation that exclusive Tf alleles may exist in two geographically diverse regions (Quinteros and Miller, op. cit.) suggests a possible means for establishing a criterion for the subspecific classification of bison.

Two other genetic loci (alcohol dehydrogenase (ADH) and malate dehydrogenase (MDH)} are also examined for polymorphism.

Materials and Methods

Sera were obtained from the Canadian Wildlife Service Pathology Unit. Hybrid sera (plains x wood) had been collected for brucella testing and had been stored at refrigerator temperatures (0-5°C). Plains

and wood bison sera had been kept frozen; however, much of it had undergone at least two freeze-thaw cycles. Many bybrid sera appeared cloudy.

Eight wood bison sera, 13 plains and 100 hybrid sera were stained for general protein. Eight wood bison, 6 plains bison and 30 hybrids were each stained for ADH and MDH after vertical polyacrylamide gel electrophoresis.

Methodology of continuous electrophoresis was conducted as outlined in Vertical Gel Electrophoresis (E-C Apparatus Co., Philadelphia, Pa.).

Serum Proteins

Seven microlitres of serum were added to each of twelve pockets in a 5.0% polyacrylamide slab gel. Anionic electrophoresis was carried out for 100 minutes at 250 volts in a 0.1M tris-borate-EDTA buffer system adjusted to pH 8.9 with boric acid. Proteins were stained in 0.2% Amido Black 10B in methanol-water-acetic acid (5:5:1) for 1½ hours and later destained in 10% acetic acid.

Malate Dehydrogenase (MDH)

Conditions for electrophoresis were the same as for serum proteins. The enzyme was stained for 4 hours using the method of Smith (1968) modified by adding

10 mg. potassium cyanide per 100 ml. of staining solution adjusted to pH 7.5 with tris.

Alcohol Dehydrogenase

Condition for electrophoresis as above. The enzyme was stained according to Brewer (1970) with the above modifications.

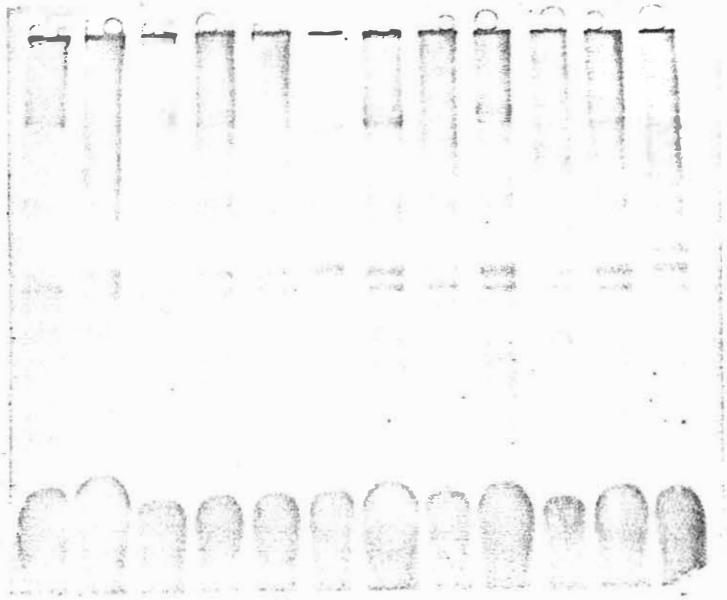
After histochemical staining of enzymes (ADH and MDH), the gel slabs were fixed overnight in a solution of water-methanol-acetic acid (5:5:1). Positions of enzyme bands were recorded and individual gels were wrapped in Saran Wrap (Dow Chemical Co.) and stored at 0-5°C for later photographic records.

Results

Serum Proteins

The system used for serum protein separation was capable of resolving up to ten or eleven protein fractions (figure 1). Variable patterns occurred in the region marked 'Tf' in figure 1.

Tf is a tentative designation for transferrins which normally occur in this region (Kubik, 1968). Protein fractions 1-5 (figure 2b) are equally spaced with relative mobilities of 0.62, 0.71, 0.81, 0.86 and



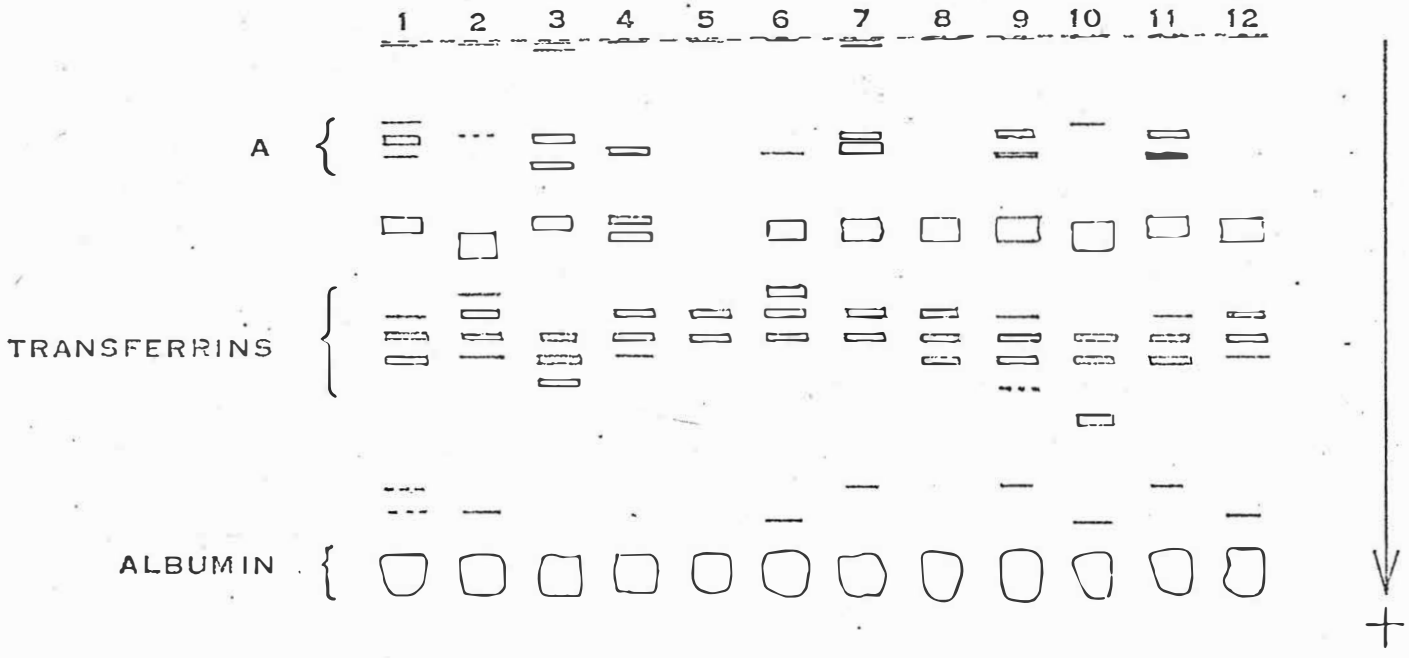


Figure 1: Electropherogram in 5.0% polyacrylamide gel of serum proteins of bison. Regions of variability are marked 'A' and 'transferrins'. Diagram corresponds to photograph.

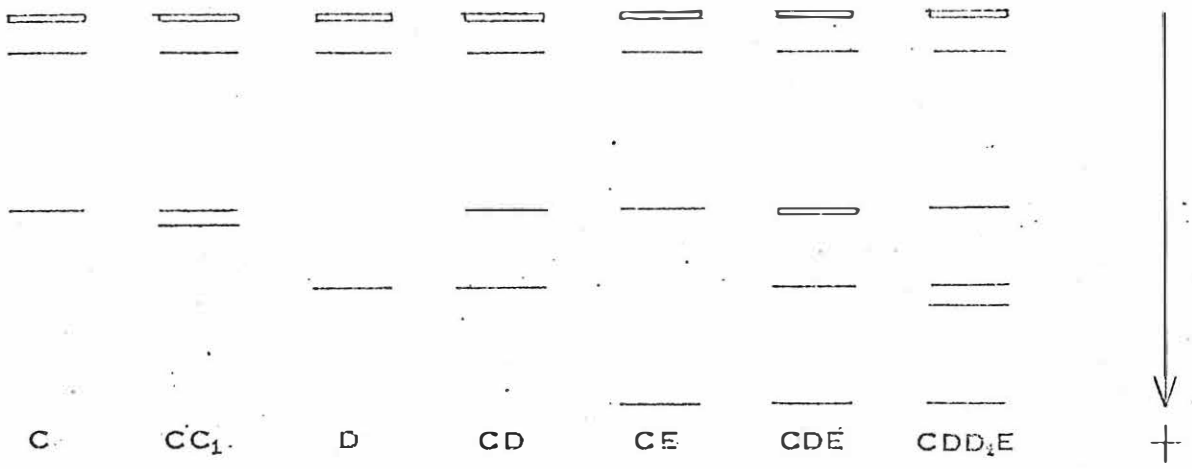


Figure 2a: Malate dehydrogenase phenotypes in bison sera.

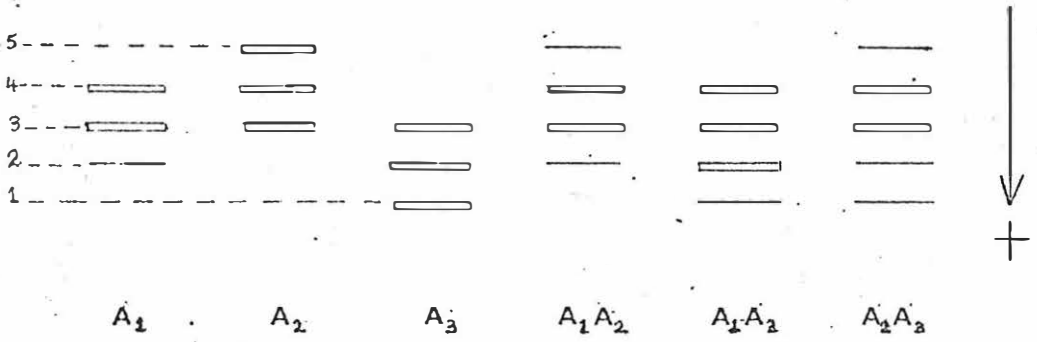


Figure 2b: Phenotypes in transferrin region of serum protein electropherogram (see also Fig. 1).

1.00 where 1.00 equals the fastest migrating Tf fraction. The Tf region exhibited six phenotypes, A_1, A_2, A_3, A_1A_2, A_1A_3 and A_2A_3 (figure 2b and 2c). Six plains bison sera which had been kept at 0-5°C exhibited phenotypes A_3, A_1, A_2 and A_1A_2 in frequencies of 0.17, 0.33 and 0.50 respectively. One hundred hybrid sera showed phenotypes A_1, A_2, A_3, A_1A_2, A_1A_3 and A_2A_3 with frequencies of 0.10, 0.26, 0.14, 0.25, 0.19 and 0.06 respectively. One rare phenotype was observed (figure 3). Of the eight wood bison sera tested phenotypes A_1, A_2, A_1A_2 and A_2A_3 occurred in frequencies of 0.13, 0.63, 0.13 and 0.13 respectively.

Eleven plains bison sera which had remained frozen until tested exhibited phenotype A_1A_3 only (figure 4).

Although not consistantly well resolved, one other region (A) also appeared to produce Heterogeneity of band mobility (figure 5). No variability existed at A, however, in sera (plains bison) which had been kept frozen until time of testing (figure 4).

Malate Dehydrogenase (MDH)

Seven phenotypes were obtained are illustrated in figure 2a and figure 6. The six plains bison sera tested showed phenotypes C, CD and D in the ratio

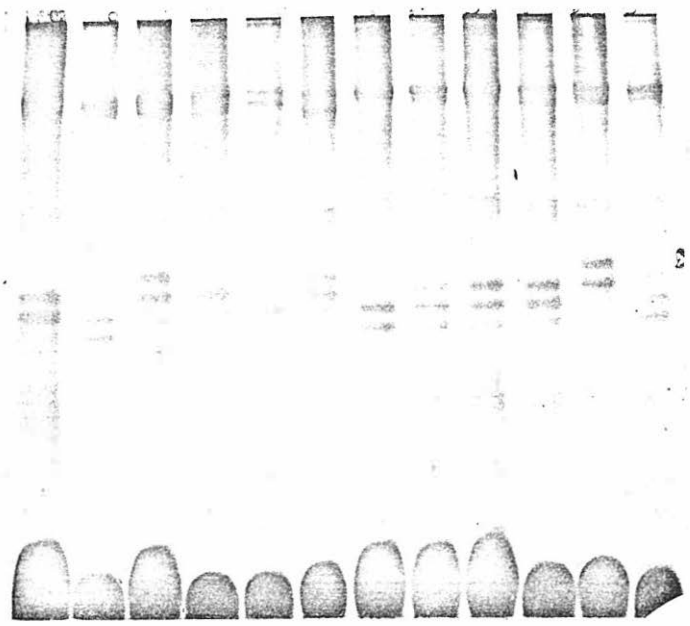


Figure 2c: Variability in the Tf region. Slots 1-12 (left to right) illustrate phenotypes A_1, A_1A_3, A_2, A_2A_3, A_1A_3, A_2, A_2A_3, A_2A_3, A_1A_2, A_1A_2, A_2 and A_3 respectively.

1:2:3 respectively. Wood bison sera exhibited phenotypes C, CD and D in frequencies of 0.63, 0.13 and 0.25. Six phenotypes were found in the hybrids occurring at frequencies of 0.53, 0.07, 0.03, 0.07, 0.23 and 0.03 for phenotypes C, D, CC_1, CDE, CE and CDD_1E respectively.

In staining the MDH sometimes achromatic regions occurred manifesting white bands on a light purple background (figure 6). These bands appeared to be variable with respect to their electrophoretic mobilities.

Alcohol Dehydrogenase

All but one of the sera samples examined showed a two band phenotype with relative mobilities of 0.57 and 0.83. One wood bison had a three band phenotype with relative mobilities of 0.57, 0.83 and 1.0 where 1.0 equals the fastest migrating band.

Discussion

A cursory look at the results from serum protein separation appears to indicate a polymorphic locus of 3 three band homozygous patterns (A_1, A_2 and A_3) and 3 four or five band heteroygous types (A_1A_2, A_1A_3 and A_2A_3). It has been observed, however, that

sera which has not been maintained below freezing will exhibit an altered transferrin pattern. Sera C (see Appendix, Page 4, U31) which displayed phenotype A_1 at the beginning of the study showed a type A_1A_2 Tf pattern after three weeks of refrigerator storage. This alteration of phenotype is thought to be the result of sialic acid residues on the Tf molecule being cleaved off upon standing at above freezing temperatures (Dr. A.A. Grunder, personal communication). This observation is born out in the results which showed no heterogeneity of transferrins which had been kept frozen. This artifactual effect of Tf pattern variation on standing can be duplicated in cattle serum by neuraminidase treatment (Stratil and Spooner, 1971). Stratil and Spooner (op. cit.) have shown that serum treated with neuraminidase, which cleaves sialic acid residues from transferrin molecules, will manifest an altered transferrin pattern. From the small sample of serum which was stored frozen it would appear that polymorphism at the transferrin locus is unlikely (in plains bison at least). This assertion is in accordance with the findings of Braend and Stormont (op. cit.) who found a constant four band electrophoretic Tf pattern throughout the animals examined. They

attributed lack of transferrin (and hemoglobin) polymorphism in Bison bison to a small sample size (113) which would not be representative of all bison. Braend and Stormont (op. cit.) suggested that genetic variation in these traits was lost during the drastic reduction of the bison population in the 1800's and there is also a possibility that bison evolved with little genetic variation in these traits. Perhaps under natural selection mutants at the Tf locus do not accumulate as rapidly as they do under artificial selection (as in cattle). That changes in the primary protein structure are not electrophoretically detectable (Shaw, op. cit.) is quite plausible. The small sample size of frozen sera (11) negates any definite conclusion as to whether this locus is polymorphic in either subspecies or their hybrids. The same may also be said of the more cathodic locus (A) which showed no variability in frozen sera. The positive identification of the Tf locus as transferrin requires confirmation by Fe^{59} labelling and autoradiography. Small sample size (of frozen sera) may have contributed to lack of polymorphism seen. Naik and Anderson (op. cit.) found three phenotypes of 6PGD. The homozygous recessive phenotype (PdAA) occurred at the frequency of 0.023 (2/86); thus

it is probable that in the present analysis, other phenotypes (of transferrin) occurring at a similar frequency were not revealed due to the small number of individuals sampled.

That MDH is polymorphic in bison appears evident from the results as seven phenotypes were observed. As more hybrids were sampled than plains or wood bison it is expected that more phenotypes would be found. A larger sample of plains bison should reveal phenotypes more similar to the hybrids than to the wood bison. As mentioned in the introduction, the hybrids resulted from an introduction of plain bison into wood bison range. This "genetic swamping" was in the order of four to one in favour of the plains bison with the result that hybrid progeny have a preponderance of genetic characters of the plains type (Banfield and Novakowski, 1960). That wood bison, which have evolved less than the plains bison (as exhibited by less specialization than the plains, Novakowski, unpublished) could display as much polymorphism as plains bison is not surprising. Although one might assume genetic invariability synonymous with morphological stability, it has been shown that Limulus polyphemus, which has changed little from its Jurassic ancestors, is polymorphic (Selander et al,

1970). Thus the degree of polymorphism may shed little light on evolutionary changes in bison. The assertion, however, that wood bison show more invariability than plains bison (or vice versa) is negated by the small number sampled. Achromatic regions in the MDH staining which results from tetrarolium oxidase* activity (Brewer, 1970) may cause artifacts in dehydrogenase banding by preventing the reduction of tetrazolium salts (Oelshlegel and Stahman, 1971). Oelshlegel and Stahman (op. cit.) found 4/6 of the tetrazolium oxidase bands in potato tubers are cyanide sensitive. Rudolph and Stahman (cited in Oelshlegel and Stahman op. cit.) found MDH staining in bean leaves unsuccessful unless sodium cyanide was added. Although potassium cyanide was added to the sera in the present study it is apparent (figure 6) that all oxidase activity was not inhibited and thus some MDH activity may be obscurred. In the frozen sera, the effect of freezing or thawing may or may not have had detrimental consequences on enzyme activity. Although the activity of some of the dehydrogenases are not altered by freezing and thawing (Brewer, 1970), some esterase loci appear to be affected (Selander et al. 1971). Sartore et al (1969) in their studies of carbonic anhydrase in cattle and American

*Note: telrazolium oxidase=indolphenyl oxidase.

bison found that there was a decrease in activity of the isozymes in region I and III. Much of the plains and wood bison sera used in this study had undergone at least a couple of freeze-thaw cycles.

Of the forty-four individuals tested, only one (a wood bison) showed a variant at the ADH locus. This may represent a real phenotypic difference or be artifactual in so far as pathological conditions in the animal may have altered the normal ADH pattern. Hudson et al (1971) have noted changes in the electrophoretic pattern of seromucoids of Rocky Mountain bighorn sheep during periods of increased parasite activity and bacterial infection. Serum isozymes of lactate dehydrogenose and alkaline phosphatase following myocardial infarction and disease of the liver respectively, have been shown to undergo changes (Latner and Skiller, 1968). As the detailed pathology of most of the bison is unknown, this factor could account for some phenotypic variations.

An assessment of individual variation and patterns of geographical variation should procede taxonomic evaluations of the two subspecies. Rasmussen (1969) points out the inadequacy of taxonomic generalizations, such as assuming a constant protein pattern for a species.

In summary, this study failed to show any genetic differences, which could be used for classification purposes, between the two bison subspecies, B. b. bison and B. b. athabascae. Any differences between subspecies would probably be a variation in gene frequencies such as in the Tf2 allele of Atlantic salmon around the British Iles (Payne et al. op. cit.). Polymorphism at the MDH and possibly the ADH loci appear to have been demonstrated. Freeze-thaw cycles, storage above freezing, tetrazolium oxidase activity, pathology and small sample size of the pure stains appear to have contributed to these indefinite conclusions.

Recommendations

1. Sera to be collected for genetic analysis should be drawn from fresh whole blood and be maintained at subfreezing temperatures until use. Several alliquots of serum from each animal should be frozen to avoid freezing and thawing of samples after each testing.
2. Transferrin, MDH and ADH loci should be re-investigated on frozen sera.
3. Confirmation of transferrin components should be made with heavy iron labelling following by autoradiography.
4. Red cells should be collected and stored (as per literature review) for investigation of red cell enzymes such as 6-PGD and G6PD (see Naik and Anderson, op. cit.).

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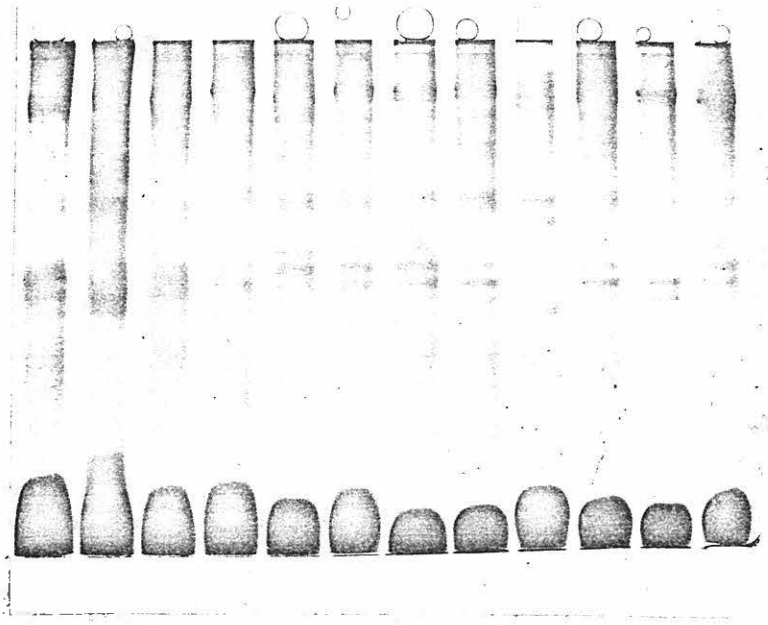


Figure 3: Rare Tf phenotype in hybrid sera (fourth slot from left).
right.

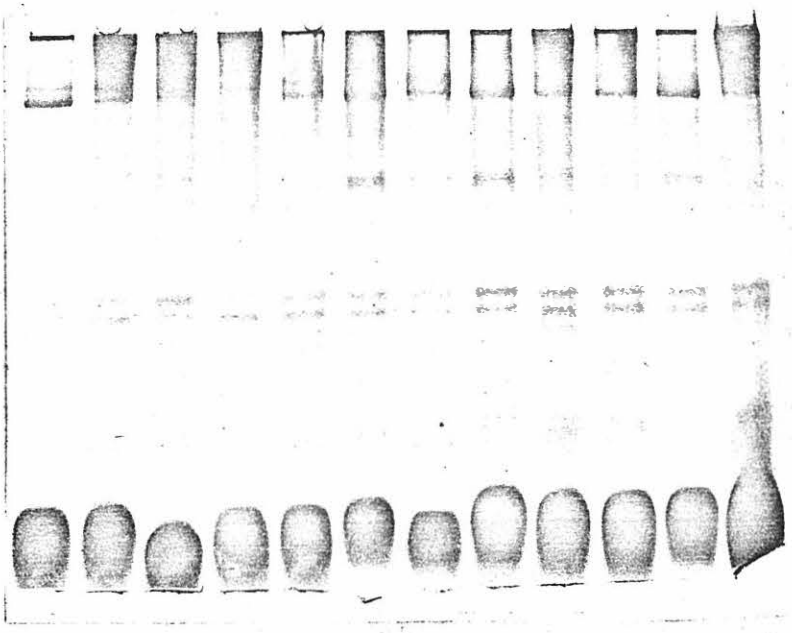


Figure 4: Sera which had been frozen (all except second slot from left) exhibited only phenotype

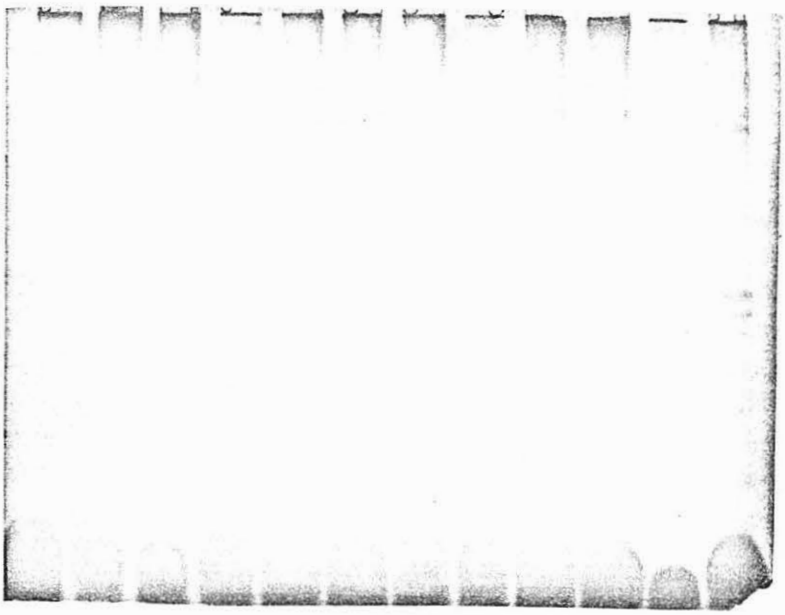


Figure 5: Heterogeneity of band mobility in A region (see also figure 1).

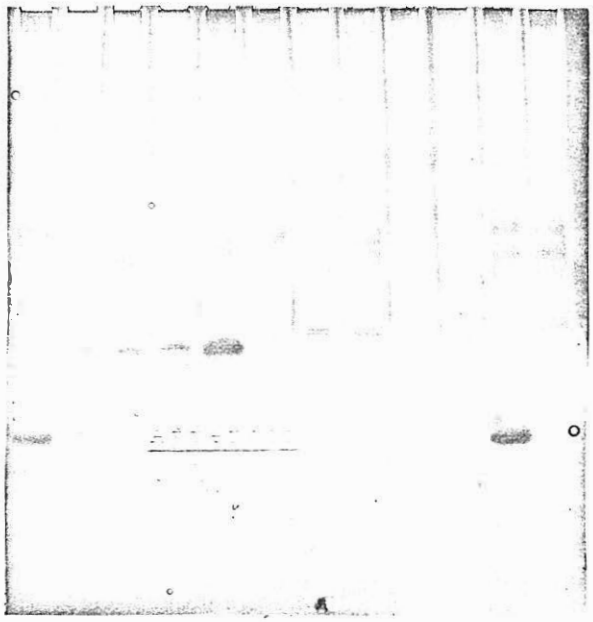


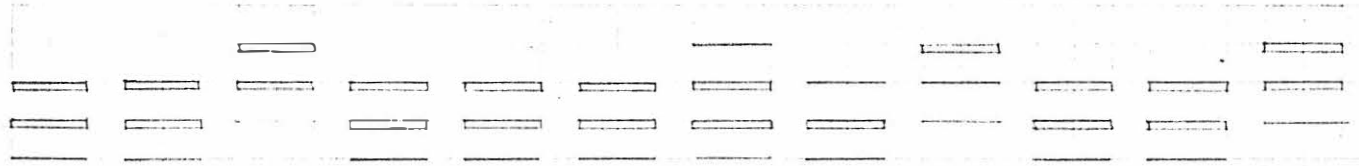
Figure 6: White bands (lower part of photograph) represent zones of tetrazolium oxidase activity in gels stained for MDH. MDH phenotypes present are (from left to right) D,CDD$_1$E,C,C,C,C,C,C,C,C,D and C.

Appendix

The following appendix consists of diagrams of the Tf locus in serum protein electropherograms and zymograms of MDH.

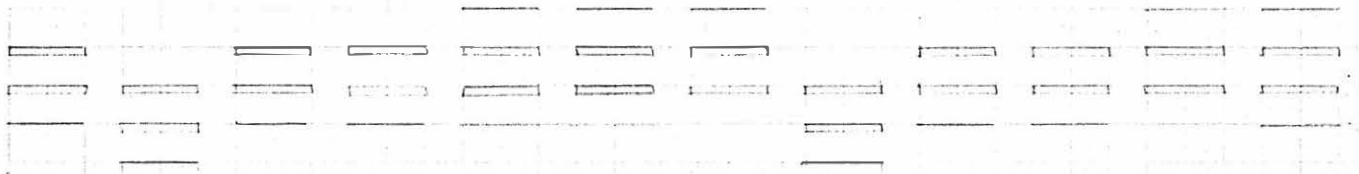
U

1	2	3	4	5	6	7	8	9	10	11	12
C	36	24	196	172	165	118	111	112	C	36	24



U 10

1	2	3	4	5	6	7	8	9	10	11	12
C	A	B	C	D	E	H	A	B	C	D	E

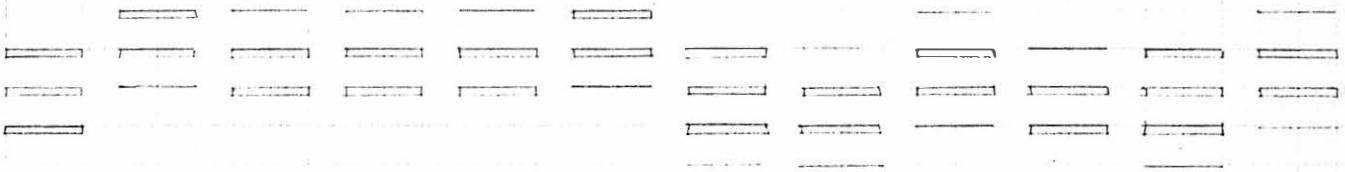


U 12

1	2	3	4	5	6	7	8	9	10	11	12
C	WB 8	A	WB 2	B	WB 7	D	WB 5	E	WB 4	H	WB 6

U 15

1	2	3	4	5	6	7	8	9	10	11	12
C	WB1	WB7	120	93	101	6	193	22	27	26	18



U 13

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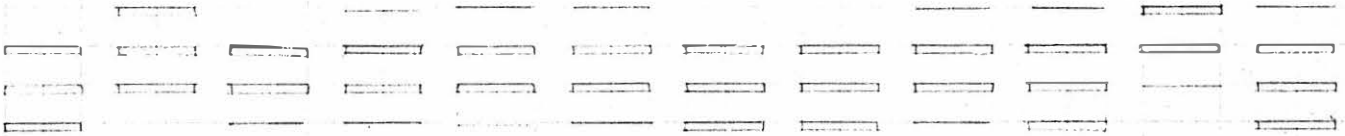
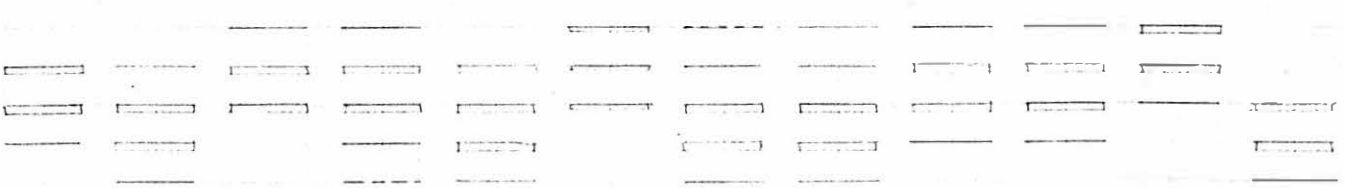
C	16	17	38	43	51	85	87	117	190	103	119



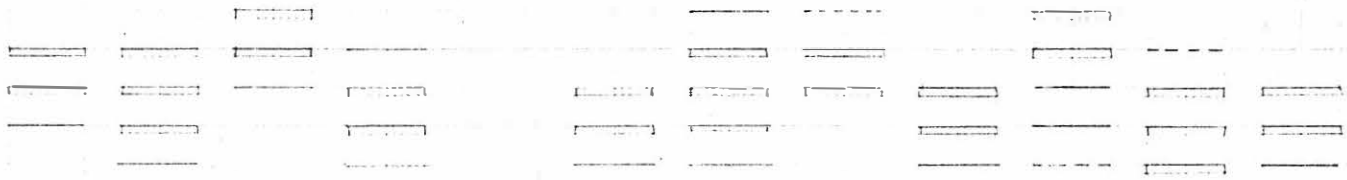
U 24

1	2	3	4	5	6	7	8	9	10	11	12
C	82	177	91	84	197	4	1	189	3	2	10



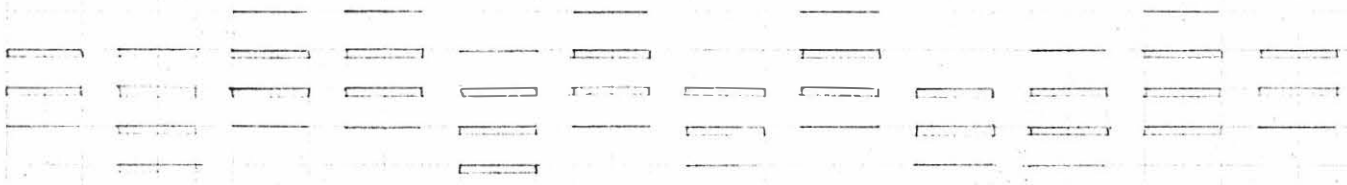
U23

1	2	3	4	5	6	7	8	9	10	11	12
C	BOVINE	106	87	83	47	71	66	56	104	108	53



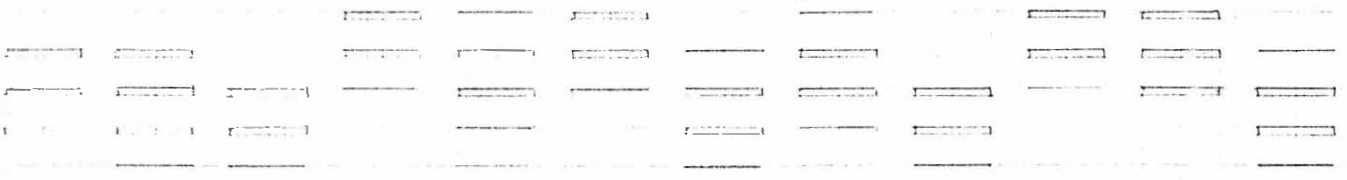
U28

1	2	3	4	5	6	7	8	9	10	11	12
C	BOVINE	17	113	185	177	166	99	159	171	183	C



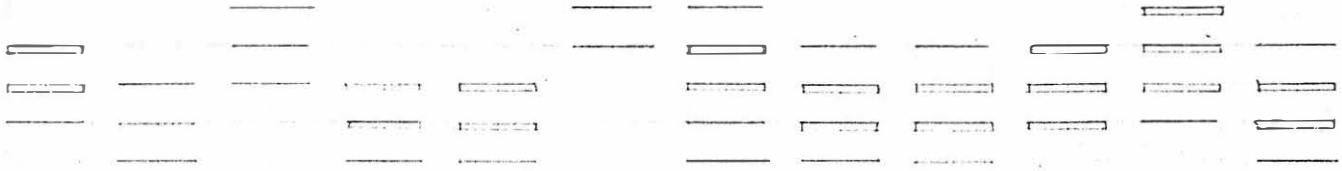
U29

1	2	3	4	5	6	7	8	9	10	11	12
C	BOVINE	153	129	141	133	137	124	152	150	148	110



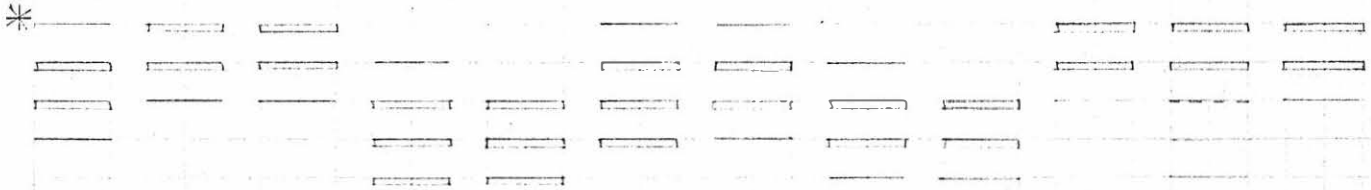
U30

1	2	3	4	5	6	7	8	9	10	11	12
C	157	122	127	131	135	139	140	147	151	155	149



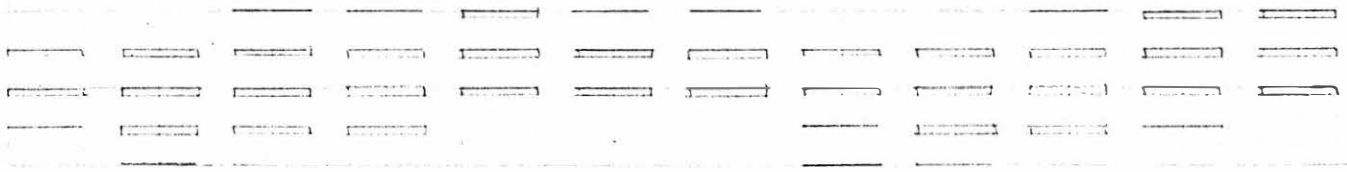
U31

1	2	3	4	5	6	7	8	9	10	11	12
C	154	123	131	126	134	138	142	146	150	156	128

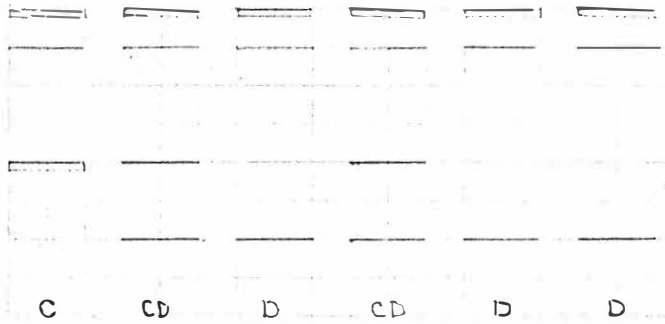


U34

1	2	3	4	5	6	7	8	9	10	11	12
C	BWINE	40	176	37	39	34	23	11	13	7	12

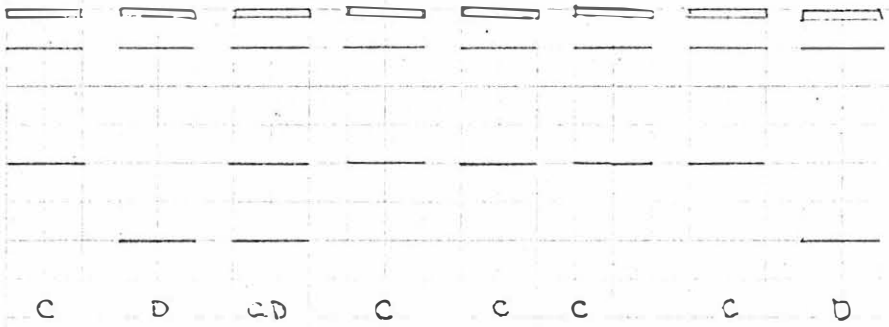


1	2	3	4	5	6
A	B	C	D	E	H



C	CD	D	CD	D	D

1	2	3	4	5	6	7	8
WB1	WB2	WB4	WB5	WB6	WB7	WB8	WB9

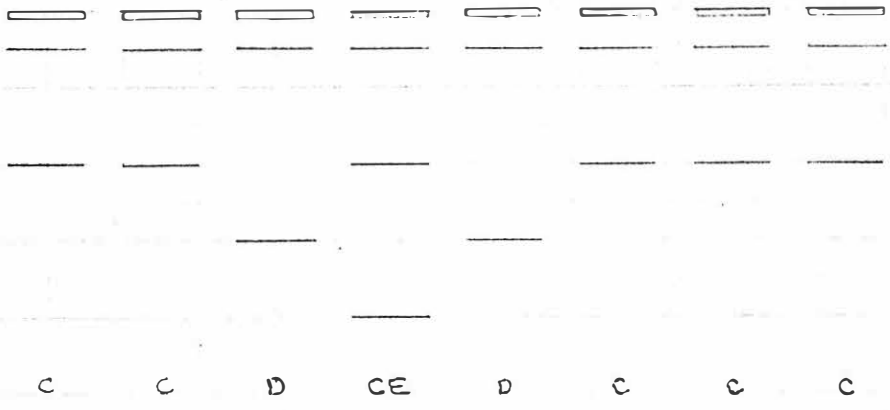


C	D	CD	C	C	C	C	D

1	2	3	4	5	6	7	8	9	10
120	96	101	6	193	22	27	26	18	5
C	C	CC_1	CDE	CE	CE	CE	CDD_1E	CE	CE

11	12	13	14	15	16	17	18	19	20	21	22
21	16	17	88	48	51	85	87	117	103	190	179
CE	CDE	C	C	C	C	C	C	C	C	D	C

23	24	25	26	27	28	29	30
19	113	185	179	166	99	173	183



C	C	D	CE	D	C	C	C