The major histocompatibility complex of African cattle.

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Abstract

An account is given of an investigation into the occurrence and nature of the bovine MHC, which is expressed as the bovine lymphocyte antigen (BoLA) system, in African and European cattle.

A series of computer programmes was written to analyse, store and retrieve the data. A new means of clustering the reactivity of the antisera was used, which did not lead to "displacement" of the cluster after each amalgamation with a serum.

The BoLA-typing was performed using a panel of antisera raised in Europe (which detected all of the internationally recognised BoLA specificities), and a panel of sera raised in Kenya during the course of the study. All internationally recognised specificities are believed to have been found in the African cattle, although some had significantly different frequencies in the two groups. Many of the European antisera which are operationally monospecific in *Bos taurus* cattle, showed extra activity in the African animals. There was evidence that two clusters (w9 and w20) could not be reliably detected in the African cattle with the reagents available.

Monoclonal antibodies were informative when used as typing reagents, particularly in the elucidation of sub-groups. The future role of such reagents in BoLA-typing seems great.

Evidence was obtained for sub-groups of w8 and w10. Five new BoLA-A locus alleles were detected by means of antisera prepared in Kenya. Two of these occurred at an extremely high frequency in African cattle, one being unique to these cattle. There was tentative evidence for MHC antigens whose expression was not controlled by the BoLA-A locus.

Two broad groups of African cattle were tested, Borans and Zebus. Borans may not have any Bos taurus blood but have been intensively selected for beef production. The frequency of some BoLA specificities, which differed between the European and Zebu cattle, was intermediate in the Borans. In other cases, the frequency of specificities in the Borans more closely resembled that of the European animals than that of the Zebus. The possibility is discussed that relatively intensive selection of the Borans has shifted their BoLA profile.

The results of this study were entirely consistent with the hypothesis that the bovine MHC is exposed to natural selection. It may therefore be of value in a programme of breeding aimed at increasing productivity while maintaining or improving disease resistance characteristics.

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Chapter 1

INTRODUCTION AND LITERATURE REVIEW

Work on several species, particularly the human and the mouse, has provided a mass of evidence that the major histocompatibility complex (MHC) plays a fundamental role in the regulation of the immune response (reviewed by Klein et al, 1983). Study of the human MHC has reached the point where decisions about diagnosis and treatment strategy of a number of diseases are influenced by the MHC genotype of the patient (Terasaki, 1980). In Europe, cattle are of great economic significance and a comparable knowledge would have considerable impact on the efficiency of the beef and dairy industries. This has prompted a number of groups to study the bovine MHC. All have so far concentrated their efforts on European cattle.

In Africa, where annual food production is declining and the population increasing (Trail et al, 1985), the value of such information would be even greater. Cattle are a vital means of generating protein, particularly in the semi-arid regions (Pratt and Gwynne, 1977). However, cattle are excluded from vast tracts of land by trypanosomiasis, while in other areas East Coast Fever causes high calf mortality, reduced productivity and precludes the use of susceptible breeds (Ristic and McIntyre, 1981; Irvin et al, 1981). If the effects of these or other diseases could be reduced by a breeding strategy based upon exploitation of certain MHC genes with given

characteristics, the increase in available food could be great.

Before an attempt to look for associations between MHC phenotype and disease susceptibility may be made, it is necessary to be able to identify the MHC products. In European cattle, the gene frequency of unidentified class I alleles (the "null allele frequency") in several breeds has a mean of about 30% (Oliver et al, 1981; Anon, 1982). In East Africa, preliminary studies showed that the frequency of the null allele was considerably higher than this and also that many sera which were operationally monospecific in Europe, contained extra antibody populations which caused reactions not due to the single specificity that the sera detected in European animals.

This study was begun with the object of improving the definition of the MHC of East African cattle using conventional serology, the only approach so-far available that will allow rapid and inexpensive screening of large numbers of animals and of applying the new reagents in a population study of the indigenous breeds.

The structure of the major histocompatibility complex

In man the MHC is expressed as the HLA system. In the mouse it is known as the H-2 system. These are the two most fully understood MHC's and they are extremely similar to each other. Their products may be divided into two major groups, class I and class II, although there are genes within the MHC which are responsible for control of some complement components and these are sometimes considered to form a third class of MHC genes (Klein *et al*, 1983).

The class I products

The serological identification of H-2 class I alleles was greatly facilitated by the microlymphocytotoxicity test of Gorer and O'Gorman (1956). This test makes use of the complement-dependent cytotoxicity of alloantisera which are specific for the products of particular MHC alleles. It is the basis of the serological tests which have been used ever since.

The class I products of every species studied are glycoproteins of much the same form. The structure of the H-2 and HLA class I products is reviewed by Ploegh *et al*, (1981). They comprise a membrane-embedded, glycosylated heavy chain in tight, but non-covalent association with a Beta-2-microglobulin molecule. The bulk of the molecule is extracellular but part of the heavy chain extends through the membrane and protrudes into the cytoplasm.

The molecular weight of the heavy chain is 44,000 to 48,000 daltons. The molecular weight of the Beta-2-microglobulin is approximately 11,500 daltons, Beta-2-microglobulin is not encoded within the HLA region (Goodfellow et al, 1975). There is significant sequence homology between the products of the HLA class I loci and those of the mouse, guinea pig and chicken (Silver, 1976; Longenecker and Mosmann, 1981; Ploegh et al, 1981). These antigens are expressed on virtually every cell of an animal (Klein et al 1983).

An outstanding characteristic of the MHC products of many species is their polymorphism. For example, the number of presently recognised alleles for the human class I loci are;

HLA-A.....23
HLA-B.....49
HLA-C.....8

(Bodmer and Bodmer, 1984a).

In respect of this polymorphism, the MHC differs from almost every other known polymorphic system in which there are one or two very common alleles and a few much rarer ones (Dausset, 1981a).

Some HLA alleles are more common than others and some are characteristic of certain populations (eg. Terasaki, 1980). The proportion of individuals who are heterozygous at the HLA A, B and D loci is greater than 80% (Bodmer and Bodmer, 1978). There is considerable

debate over the origin of this polymorphism but it is becoming accepted that it is a reflection of the function of the MHC (Zinkernagel and Doherty, 1979).

The products of the class I loci are highly antigenic and they are responsible for rapid allograft rejection (Klein et al, 1983). They have been shown to be involved in cellular interactions, particularly those involving cytolytic T-cells (CTL) (Doherty et al, 1976).

The class II products

The class II products were first detected by the use of the mixed lymphocyte culture (MLC) test (Bach and Hirschhorn, 1964; Bach and Voynow, 1966). They are thus sometimes known as lymphocyte defined (LD) loci. In man, the region which controls the response in an MLC was named HLA-D (Thorsby and Piazza, 1975). Subsequently, a series of antigens closely associated with HLA-D was detected serologically. These were known as HLA-DR (for D related) antigens (van Rood et al, 1977).

The HLA-D region has since been shown to comprise at least three regions DP, DQ and DR, each of which contains several loci (Bodmer and Bodmer, 1984a). The products of the DR and DQ regions are defined both serologically and by MLC response. Those of the DP region are only detected by MLC (Bach, 1985). Monoclonal antibodies have proved to be of particular value in the serological definition of the HLA class II alleles (Bodmer and Bodmer, 1984a).

The tissue distribution of the HLA class II products is different from that of the class I products in that they are expressed on the surface of a limited range of cell types, particularly macrophages, B-cells, and to a lesser extent, T-cells (Hammerling et al, 1974; McDevitt, 1978). Under certain conditions however many cell types can be induced to express class II products, for example human T-lymphocytes will express such antigens at a density comparable to that of B-cells following stimulation by antigens or mitogens (Evans et al, 1978, Zier and Zmijewski, 1984).

Like class I products, the class II products show considerable polymorphism. By analysis of MLC, a total of 25 class II alleles have been described and 16 antigens have been serologically defined (Bodmer and Bodmer, 1984a).

The class II products seem to have a particularly important role in antigen presentation (Klein et al, 1981), which is in contrast to class I products which exert their greatest influence on CTL.

MHC-controlled phenomena

There is a great deal of evidence that the MHC has an important function. Resistance or susceptibility to many diseases is related to the MHC phenotype of the individual, particularly in chickens (Collins et al, 1977; Longenecker and Mosmann, 1981; Collins and Zsigray, 1984) and man, where findings of great significance to genetic counselling and clinical strategy have been made (reviews in Terasaki, 1980; Bodmer and Bodmer, 1984a). It was proposed by Dausset (1981a) that each MHC haplotype possesses a range of characteristics of resistance and susceptibility. For example the HLA-A3, B7, DR2 haplotype is associated with susceptibility to multiple sclerosis and protects against insulin-dependent juvenile diabetes.

Further evidence that the study of BoLA is crucial to an understanding of the bovine immune system is provided by the role played by the MHC of other species in a range of cellular interactions. For instance, H-2 class I products were shown to influence the ability of cytolytic T-cells to destroy virus-infected cells (Doherty et al, 1976) and TNP-modified cells (Shearer et al, 1975; Forman, 1975). Similar phenomena were subsequently shown with several species and several viral pathogens (Zinkernagel and Doherty, 1979).

Class II products have been shown to control the collaboration between murine T and B cells, which is frequently required before B-cells can begin to produce an

antibody response (Kindred and Shreffler, 1972; Katz et al, 1973a; 1973b). Similar interactions have since been suggested in the chicken (Vainio et al, 1984). Class II products also restrict antigen presentation by macrophages (reviewed by Munro and Waldman, 1978).

The evidence that the MHC is exposed to natural selection

If the MHC is of as great significance as its associations with disease resistance and its involvement in a range of *in vitro* phenomena suggest, then it would be expected to show evidence that it is exposed to natural selection. There is a great deal of such evidence.

The polymorphism of the MHC

Possibly the most striking feature of the MHC is its polymorphism. Does this reflect selective pressures which favour a wide variety of alleles, or is it the result of the random drift of genes with products whose precise form is irrelevant to the fitness of the individual carrying them? There are a limited number of ways in which polymorphism may be maintained in a population.

Neutral genes

One of these is the effect of neutral genes (Lewontin, 1974; Nei, 1975). If the survival value of one allele is exactly the same as that of another allele, then their frequency in a population will be determined by chance alone. This situation may arise either because different alleles produce equally effective products, or because the locus has no function.

Ohta (1976) discussed the effect of multiple, very slightly deleterious mutations in the maintenance of polymorphism, and concluded that mutations which have an

effect on the fitness of an individual of less than 1% may play a part in the maintenance of polymorphism. This model however applies to large numbers of rare alleles and could not account for the large numbers of alleles with relatively high frequencies that are seen in the MHC.

Disruptive selection

Another way in which polymorphism may be maintained is by disruptive or apostatic selection. This is selection against the majority phenotype so that the fitness of an allele will not depend solely upon its own characteristics, but also upon its frequency in the population. This type of selection has been shown to occur in a number of predator/prey interactions where a predator acquires an increased ability to detect the common phenotype (Clark, 1969; Allen, 1976). A population which is polymorphic for a characteristic which forms part of the predator's "searching image" is less heavily predated than one which is monomorphic (Moment, 1962; Croze, 1970). The nature of the resultant polymorphism will depend on the nature of the selecting factors and the nature of the alleles which are being selected. The crucial aspect of this type of selection, and the way in which it differs from those discussed below, is that it will tend to produce a specific, divergent population structure. The survival value of each allele is influenced by the presence of every other allele in the population.

Heterozygote advantage

Heterozygote advantage is another phenomenon which may result in a balanced polymorphism. This is seen where the heterozygote has a selective advantage over the homozygote.

Although population studies (Degos et al, 1974) have not produced clear answers, there is evidence that heterozygote advantage occurs in HLA. The frequency of homozygotes at the HLA-A, B, C and DR loci at the ninth HLA workshop was significantly below the level that would be expected if there was no heterozygote advantage (Klitz et al,1984).

Differential chances of successful reproduction

Polymorphism may also be maintained by non-random mating or by interactions between the mother and foetus which favour differences between the haplotypes of the two. There is evidence that mice of different MHC types preferentially mate with each other (Yamazaki et al, 1976; 1978) and it is clear that mice can detect H-2 differences between congenic individuals by odour alone and that they can learn to distinguish recombinations within the H-2 region (Boyse, 1983). Rosenberg et al (1983) reported the results of investigations into the HLA type of mating couples and found that there was a greater frequency of couples with identical HLA-A and B types than would be expected by chance. However, this may well be due to the influence of race and ethnic origin.

There is however, evidence (reviewed by Johnson, 1982) that spontaneous abortions of unknown cause occur more often when the parents are of very similar HLA type that is, they share more than 3 alleles at HLA-A, B, C and DR. This would provide a mechanism for heterozygote advantage. An investigation by MacQueen and Sanfilippo (1984), based on the result of HLA-typings at one American laboratory, failed to support this suggestion but their study was based on relating the number of children per family to the HLA type of their parents and this would be expected to be an insensitive approach, particularly in America where family size is a very poor measure of fertility.

Linkage disequilibrium

Further evidence that the MHC has been exposed to selection is provided by the high linkage disequilibria which are characteristic of many HLA haplotypes (Grange *et al*, 1984) For instance, in some populations, the HLA A1/B8 haplotype frequency is more than four times greater than the product of the A1 and B8 gene frequencies (Bodmer and Bodmer, 1978).

Many alleles show this sort of linkage disequilibrium. Many linkage disequilibria are characteristic of certain populations (Pickbourne *et al*, 1978) and some alleles are more often in linkage disequilibrium than others (Bodmer and Bodmer, 1978). It is also clear that alleles of the HLA-B and HLA-C loci are

more often in linkage disequilibrium than alleles of other loci (Bodmer and Bodmer, 1978). Bodmer and Bodmer (1978) calculate that 66.5% of B/C haplotypes are due to linkage disequilibrium, while 21.7% of A/B and 34.3% of B/DR combinations result from this phenomenon.

It is known (Jennings, 1917; Robbins, 1918) that in a random mating population at equilibrium and under no selective pressure, the linkage disequilibrium between any two loci will be zero. If it were not zero, then it would approach zero under such conditions at a rate of 1-r per generation, where r is the recombination fraction of the loci.

This being so, any linkage disequilibrium in a population must be the result of selection, non-random mating, or an effect of linkage which will eventually vanish. Since the value of r for HLA loci is small (Bodmer, 1984), any of the above factors will rapidly lead to significant linkage disequilibrium. Many A/B and B/D combinations are found in separate Caucasoid populations which diverged more than 5,000 years ago (Bodmer and Bodmer, 1978). This is strong evidence that these disequilibria result from selection, since 5,000 years would be more than enough time for these effects to vanish if they had arisen by chance and were not maintained by selection.

The function of the MHC

The polymorphism, linkage disequilibria and species homology of the MHC appear to provide clear evidence that the MHC has been under strong selective pressure and therefore probably fulfills an important role. The attempts to explain this role as a means of preventing antigenic mimicry by pathogenic organisms were never well argued (reviewed by Longenecker and Mosmann, 1981) and such effects, if they exist, are not today considered significant. There is today only one group of theories which begins to suggest a function for the MHC which can account for its form, and these centre around the role of the MHC in interactions between cells of the immune system.

T-cell interactions

The demonstration (Doherty and Zinkernagel 1974; Zinkernagel and Doherty, 1974a) that the ability of immune T-cells to kill virus infected target cells is MHC restricted, led to speculation that the polymorphism of the MHC is a result of the differential ability of the immune system to respond to certain viral antigens when they were associated with certain MHC antigens (Doherty and Zinkernagel, 1975; Zinkernagel and Doherty 1974b). Early interpretations of this phenomenon invoked class I-linked immune response (Ir) genes (reviewed by Adams and Brandon, 1981). However there is no need to look beyond the presently recognised class I products themselves to

account for differences between individuals in their CTL/target cell interactions.

The nature of the T-cell receptor, which makes the recognition of pathogen antigen in conjunction with an MHC product, has been the subject of much debate (reviewed by Matzinger, 1981). It is becoming widely accepted that one receptor is responsible for the detection of a single, pathogen-modified, self MHC antigen (or a non-self MHC product alone in the case of alloreactivity) (reviewed by Teale et al, 1985a). Essentially then, the MHC is being seen as a means of marking changes in the antigenic nature of a cell.

Since the basic structure of all MHC class I products is the same (Ploegh et al, 1981), it is not surprising that a non-self MHC antigen causes cytotoxicity in the same way as an altered self antigen. Which of these was the primordial role of the MHC is an interesting question. There is evidence of an MHC-like system in sponges, which may be analogous to the ancestral MHC, the function of this system appears to be the prevention of natural allografts (Curtis et al, 1982; Scofield et al, 1982). However, in modern vertebrates alloreactivity probably has no natural function.

This model requires that different pathogen antigens are more readily seen against some MHC "backgrounds" than others. There is convincing evidence for this in the mouse where, for example, the ability of a strain to

respond to vaccinia virus and lymphocytic choriomeningitis virus is influenced by genes of the class-I region (reviewed by Zinkernagel and Doherty, 1979). This hypothesis does not itself account for the conservation in evolution of the MHC products and it remains possible that the products of the MHC perform a greater role than simply acting as markers of infected or aberrant cells. The model does however explain the polymorphism of the MHC.

The response of the MHC to selection

If it is accepted that each set of MHC alleles confers a particular set of disease resistance characteristics onto the individual bearing them, then a number of predictions may be made about the expected appearance of the MHC in a given population.

Populations will tend to become polymorphic for MHC alleles because an individual will have a greater chance of surviving a given disease if it is heterozygous. Under a stable disease challenge, heterozygote advantage alone would tend to produce a population of individuals which are heterozygous for the alleles which confer greatest advantage in the face of diseases which are exerting the greatest selective pressures. Those few alleles would become fixed and less favourable ones would be lost.

However, most diseases exert a selection which is frequency dependent. Under such a challenge, an individual in a population which is largely composed of susceptible members will suffer to a disproportionately

greater extent than an individual in a population with few such members. This is because many diseases require rapid transmission between individuals to persist. This is particularly true of diseases with a high mortality since the pathogens must find a new host rapidly. Such diseases obviously also represent powerful selective pressures.

Given a set of diseases which select in a frequency dependent manner and given alleles which have different characteristics of resistance to each, the resulting population structure would be a mosaic of different alleles. The exact form of the mosaic would depend on the nature of the disease challenge, the number of challenging diseases and the selective pressure that each exerts.

It is not surprising that most of the associations seen between MHC and disease involve rare diseases and diseases of old age (reviewed in Albert et al, 1984). This is further evidence that the MHC does play a part in determining resistance characteristics. If each allele confers a characteristic set of resistances and susceptibilities, then when one allele became fixed in a population due to its beneficial effects it will carry with it a set of deleterious effects. These effects would become masked by a set of genes, which would accumulate around it in high linkage disequilibria to produce the optimal set of characteristics. The only deleterious effects of an allele which would not in time acquire such a set of linked genes are those which are not exposed to

selection. Such effects are those which appear after reproductive age or which very rarely appear.

Thus the explanation of MHC function based on the model of T-cell killing explains very well two of the most striking features of this region, its polymorphism and its linkage disequilibria. It also suggests that associations between MHC and a powerfully selecting disease will easily be seen only in those populations which have not been exposed to the disease for many generations. This is because MHC genes are inherited in large closely-linked groups and if each product has a given set of characteristics then it will be very difficult to determine the significance of each for any one disease by means of a population study. The only study that might have been expected to overcome this problem (de Vries, 1979) did show evidence of such associations despite very unfavourable experimental conditions. The ideal experiment would involve the large-scale exposure of populations of known MHC type to a novel disease challenge. Obviously there would be some ethical difficulties in doing this with a human population. However, in East Africa, European cattle are frequently exposed to an environment that their ancestors have never experienced. This would seem to present a unique opportunity for this type of experiment. It was one of the main reasons for embarking upon this study.

There is good reason to believe that an investigation of the bovine MHC may ultimately be of value to cattle

breeding programmes by identifying BoLA alleles with given disease resistance characteristics. An understanding of, and an ability to identify, BoLA alleles is also essential to many investigations of the bovine immune system. An example is in the study of immunity to Theileria parasites where there is growing evidence that CTL are important in the immune response (Morrison et al, 1985).

The MHC of other species

As well as mice, men and cattle, an MHC has been described in the chimpanzee (Balner et al, 1971a; 1971b; 1974), the rhesus monkey (Balner et al, 1971c), the guinea pig (Ellman et al, 1970; Schwartz et al, 1976), the goat (van Dam, 1981), the rabbit (Tissot and Cohen, 1972; Chai, 1974), the rat (Palm, 1964; Stark et al, 1967), the pig (Vaiman et al, 1970; 1972), the dog (Vriesendorp et al, 1971; 1972), the chicken (Longenecker and Mosmann, 1981; Simonsen et al, 1982), the hamster (Palm et al, 1967), the sheep (Ford, 1974; Millot, 1978; Stear and Spooner, 1981) and the horse (Lazary et al, 1980a and 1980b; Mottironi et al, 1981).

These early studies emphasised homology of the MHC between species. However, recently evidence has appeared that the MHC's of some species are significantly different from the human and murine systems. It has been shown that although the Syrian hamster has at least two loci which express antigens homologous to the class I products of other species (Darden and Streilein, 1984), these products are monomorphic, or at least non-antigenic to conspecifics (Duncan et al, 1977 and 1980). This monomorphism may explain the inability of Zinkernagel and Doherty (1979) to show MHC restricted T-cell killing in the hamster. Ohno and Wallace (1983) suggest that the monomorphism of the hamster implies that the MHC is redundant and that its conservation in other species is a result of chance. However, the hamster is unusual in that it leads a

solitary life and is exposed to little or no selection by viruses (Streilein *et al*, 1984) and it may be that these unusual conditions allowed a monomorphic population to survive following a severe population bottleneck. A species which has a similar ecology, the mole-rat, has been shown to have a highly polymorphic MHC (Nizetic *et al*, 1984), but there may be significant differences in the disease challenge faced by the two species and in their ancestry.

The domestic cat shows evidence of a monomorphic class I MHC (Pollack *et al*, 1982) and the cheetah has been shown to be tolerant of allografts and also to have dramatically less polymorphism at many loci other than the MHC (O'Brien *et al*, 1985). It may be that this is due to recent bottlenecks in both populations, but it is interesting that two species of cat should both show an unusual MHC and also that both are particularly susceptible to viral pathogens (Pollack *et al*, 1982; O'Brien *et al*, 1985). It would seem extremely worthwhile to examine the MHC, and other loci, in more members of the *Felidae*.

Other species which show evidence of unusual MHC expression are the dog, which may have only one class I locus, limited polymorphism and a broad tissue distribution of class II products (van der Feltz and Ploegh, 1984) and the rabbit, where there is good evidence of a single class I locus (Marche *et al*, 1985).

However, despite these differences there does appear to be an MHC in all mammals with products which are highly homologous to the human and murine products and even amphibians (Xenopus) show such molecules (Flanjnik et al, 1984). The differences between species should not however be ignored for they may provide valuable clues to the function and evolution of the MHC.

The bovine MHC

The first evidence for an MHC in cattle was the demonstration of anti-lymphocyte activity in the sera of cattle which had received whole bovine blood innoculations (Borovska and Demant, 1967). Subsequently, McGary and Stone (1970), Ostrand-Rosenberg and Stormont (1974) and Folger and Hines (1976) reported allogeneic anti-bovine-lymphocyte sera.

The bovine class I products

In a brief report Schmid and Cwik (1972) claimed to have demonstrated the presence of 28 lymphocyte and 37 granulocyte antigens on cattle leukocytes. They used typing sera produced by immunising cattle with cattle lymphocytes. Iha *et al* (1973) reported the cross-reactivity of cattle-anti-cattle lymphocyte sera with HLA and other human antigens. This suggested that the sera were directed against the bovine MHC since many anti-MHC sera have been found to cross-react with MHC antigens in several species (Ivanyi, 1981).

A small population study was made by Caldwell *et al* (1977) who reported the results of testing lymphocytes of 103 Holstein cattle against 11 alloantisera. Four of these sera were made by lymphocyte innoculation and seven were parous sera. They suggested that cattle lymphocyte antigens were highly polymorphic and heritable.

Caldwell and Cumberland (1978) presented evidence

that one of their clusters of typing sera appeared to detect a specificity which was more common in a herd of cattle with a high incidence of ocular squamous cell carcinoma than in a herd with a low incidence. However this difference does not persist when numbers of cattle showing lesions are compared with unaffected animals. There have been no further reports of such an association and it seems that the differences in antigen frequency between the herds were unrelated to this carcinoma. Comparison of almost any two herds will yield significant differences in BoLA antigen frequency (Oliver et al, 1981).

A strong argument that the antigens on lymphocytes which were detected by sera made by alloimmunisation were indeed the bovine MHC was made by Spooner et al (1978). They pointed out that the associations between MHC and disease susceptibility in other species provide a strong incentive to conduct this work in such an economically important group as cattle. They set out to produce highly specific alloantisera in order to simplify the interpretation of the results. They described, for the first time, the production of antisera by reciprocal skin grafts between dam/calf pairs. In this way they were able to reduce the number of foreign antigens to which the recipient was exposed, since half of the graft's genome was identical to that of the recipient. They produced high titre antisera (most were between 1/32 and 1/256, whereas most parous sera have titres below 1/32). These

workers also made extensive use of lymphocyte absorption to produce a panel of "operationally monospecific" antisera. Using a panel of 70 sera in one study and 35 in another they examined the segregation of 18 antigenic factors in 249 half sibling families. These families showed segregation of the 18 factors as co-dominant alleles at a single locus. A number of factors appeared to be inherited together and it was suggested that this may be due to linked loci but it is possible that it was due to cross-reactive antisera.

Amorena and Stone (1978) describe the use of 67 anti-lymphocyte antisera. They assigned each serum to one of 11 groups on the basis of their reactions with cells from over 900 cattle of 60 sire families. They concluded that each of the 11 groups was detecting a single antigen and that the antigens were controlled by the alleles of a single locus. They proposed to call this locus the BoLA-A locus. They investigated the distribution of these alleles in 931 cattle of 5 dairy breeds and reported differences in the frequency of alleles between breeds. They performed skin grafting experiments on three cows which were matched with respect to these 11 alleles. Although the details of rejection times are not reported, rejection was delayed in BoLA-matched cattle by comparison with BoLA mismatched cattle.

Further evidence that the BoLA system is highly polymorphic was provided by Caldwell *et al* (1979). Their report, the only published work on the MHC of *Bos indicus*

type cattle, showed a higher frequency of undetectable products in Brahman/Hereford crosses than in any of the five Bos taurus breeds tested. These authors also referred to the first international meeting of laboratories working on the bovine MHC, which was held in France in 1977. This meeting was the first in a series which has contributed enormously to the understanding of the bovine MHC by allowing laboratories to compare the performance of their sera and to ensure a standardised nomenclature.

Following the meeting in France, the first international BoLA workshop was held in Edinburgh in 1978 (Spooner et al, 1979a). Nine laboratories contributed a total of 249 sera which were tested in Edinburgh against a panel of 130 cattle of 21 breeds. The reactions of the sera were clustered by various methods and 11 of these clusters were assigned workshop specificities, prefixed by "w" to show their provisional nature. The agreed specificities were:

w1	12 animals	detected by	5 antisera
w2	36 animals	detected by	8 antisera
w5	9 animals	detected by	7 antisera
w6	40 animals	detected by	9 antisera
w7	12 animals	detected by	4 antisera
w8	15 animals	detected by	11 antisera
w9	5 animals	detected by	3 antisera
w10	18 animals	detected by	7 antisera
w11	7 animals	detected by	6 antisera
w16	2 animals	detected by	6 antisera
w20	13 animals	detected by	3 antisera

By this system none of the animals tested had more

than two specificities. 11% of them had no specificity, 48% had one and 41% had two. Since they were not a random selection, no attempt was made to compare frequencies of the specificities between breeds.

The workshop also showed that the way in which the lymphocytotoxicity test is performed affects the result. Many sera were made by laboratories using slightly different test techniques to those used in the workshop, particularly in the amount of rabbit serum used as a source of complement. It is also clear that different batches of complement have different characteristics, a fact which has also been reported in relation to HLA-DR typing (Middleton, 1981). These variables may explain the poor repeatability of the reactions of many sera, and the fact that only 75% of sera gave any reaction at all. This was one of the reasons why the second workshop exchanged cells rather than sera. The meeting decided that the putative genetic region controlling the expression of these antigens be called the BoLA (bovine lymphocyte antigen) region.

A study of the response of cattle to skin grafts was made by Spooner *et al* (1979b). It showed that grafting is a reliable means of producing high titre antisera and that the main activity of an antiserum produced by grafting is directed against a BoLA specificity of the donor. This report also illustrates the value of lymphocyte absorption of antisera to demonstrate operational monospecificity.

Absorption analysis of 18 sera which detected seven of the agreed BoLA specificities was carried out by Spooner et al (1980). All clusters except w9 showed evidence of sub-groups. That is, they contained sera whose reactivity was broader than most other sera of a cluster but which were still operationally monospecific. It was not clear whether these "tails" were due to true sub-groups detected by the less reactive sera, or to polyspecificity in the more reactive sera. Another possible explanation of a sub-group is the presence of an allele at a second locus in high linkage disequilibrium with the allele defined by the narrower sera. The extensive cross absorption studies of these workers did however, strongly support the conclusions of the BoLA workshop in the assigning of specificities to these clusters.

Spooner and Brown (1980) reported the results of the BoLA typing of cell-lines infected by the protozoan parasites Theileria parva and Theileria annulata. They showed that the BoLA types of the line donors were identical to those of the derived cell-lines and that the BoLA type of the lines remained constant with time and with the multiplication of the cells. There was one exception, which was subsequently shown to be due to the presence of a minor lymphocyte population in a chimaera (Teale et al, 1983).

A family study of seven breeds (Oliver et al, 1981) provided further evidence that the 11 BoLA specificities

were in fact alleles at a single locus. 142 matings from 15 bulls provided evidence that there was a significant difference between the frequency of these alleles both between breeds and between sexes. Differences between breeds are not surprising since European cattle breeds are genetically rather isolated from each other. The differences between sexes is harder to explain, but it may well be that the males were less closely related to each other than the females.

In 1980 the second international BoLA comparison test was held in Wageningen, Holland (Anon, 1982). Sixty lymphocyte preparations from USA, 60 from Edinburgh and 24 from France were distributed to five laboratories for testing. 130 sera from five USA laboratories were tested in Texas and four European laboratories tested their own 232 sera, a total of 362 sera. The results were analysed independently by two laboratories. This meeting confirmed the presence of all of the specificities agreed at the first workshop except w1, which was however allowed to stand. In addition, four new specificities were defined:

w3	5 animals defined by	3 sera
w4	7 animals defined by	2 sera
w12	10 animals defined by	3 sera
w13	4 animals defined by	4 sera

Two sub-groups of w6 were also defined:

w6.1	8 animals defined by	5 sera
w6.2	11 animals defined by	3 sera

In this test 12% of the cells had no specificities,

45% had one and 43% had two. The agreement between the results of the two workshops was extremely good where cells had been tested in both. The second workshop did not provide any evidence of a second class I locus. This is perhaps surprising and it may reflect a real difference between the BoLA system and the MHC of mouse and man. Many laboratories tested sera of narrow specificity which did not appear to cluster with sera from any other laboratories and so could not be considered to define a specificity. It may be that some of these were detecting antigens controlled by a second locus. The fact that no other laboratories produced sera which clustered with them may be because the antigens are more highly polymorphic, of restricted geographical distribution, are poorly antigenic, or any combination of these.

There is strong evidence that the BoLA antigens described by the above reports are the bovine equivalent of the HLA and H-2 class I antigens. In addition to their polymorphism, the cross-reactivity of anti-BoLA antisera with HLA antigens (Iha et al, 1973) and the effect of BoLA matching on graft rejection times (Amorena and Stone, 1978), there is evidence that the biochemical structure of the BoLA-A antigens is similar to that of the MHC antigens of other species (Hoang-Xuan et al, 1982a). This report showed that the structure precipitated by an anti-w16-antiserum was a 44,000 molecular weight glycoprotein in non-covalent association with a smaller polypeptide of molecular weight 12,000.

There is also evidence that the BoLA products are of importance to the immune response of cattle. It has been shown (Teale, 1983; Dolan *et al*, 1984) that the anti-*Theileria* immunogenicity of *Theileria* infected cell lines correlates with the degree of sharing of BoLA-A locus products between cell line and recipient. The mechanism of infectivity of this parasite is poorly understood (reviewed by Irvin *et al*, 1981) and the interpretation of this observation is not easy. Lowered immunogenicity may result from a simple rapid graft rejection phenomenon. Further proof that the BoLA antigens are important in the immune system has come with very clear evidence (Morrison *et al*, 1985) that CTL from cattle undergoing challenge by *Theileria parva* have a cytotoxicity which *in vitro* is restricted by products of the BoLA-A locus.

There is some preliminary evidence of an association between haemolytic complement levels and BoLA type (Spooner and Lie, 1982). There is also evidence of close linkage between BoLA w16 and the M′ red-cell antigen, this appears to persist in different European breeds and recombinants may have been observed (Leveziel, 1982).

An important addition to the understanding of BoLA has come with the demonstration that at least some of the antigens detected by workshop agreed clusters can act as targets for MLC-generated CTL (Teale *et al*, 1985a; 1985b). This is the case with the class I alleles of other species (eg. Nabholz *et al*, 1974) and thus the belief that the

BoLA class I alleles are the equivalents of those of HLA and H-2 is supported and the definition of the workshop specificities is confirmed. While BoLA-specific CTL cannot be considered as routine typing reagents they will certainly provide an extremely powerful means of analysing the validity of serologically defined clusters and suggesting means of improving typing reagents. These cells have since been cloned and maintained in culture for long periods (Teale, personal communication) and can provide a unique source of monoclonal bovine-anti-bovine reagents.

THE BOVINE CLASS II GENES

The evidence for the presence and nature of a bovine class II locus is much more limited than that for the class I locus. Usinger et al (1977) demonstrated a bovine mixed lymphocyte response. Spooner et al (1978) reported groups of class I-matched full siblings which were mutually non-reactive in the MLC while they were highly stimulated by their dams. Later, Usinger et al (1981) confirmed that the locus controlling the MLR (which they called the BoLA-D locus) was closely linked to the BoLA-A locus. This was done by the use of large, full sibling families obtained by embryo transplantation. They showed that full siblings which shared class I alleles also shared class II alleles (that is they were mutual non-stimulators in the MLR). This suggests linkage between the loci, but until more data are collected and

recombinants identified, it is not possible to be more precise about their relative location.

There is evidence (Newman et al, 1982) for the existence of antigens which appear to occur predominantly on bovine peripheral blood B-cells. This was obtained by the simultaneous use of a vital stain to identify dead cells and a fluorescent label to identify cells bearing immunoglobulin on their surface.

An attempt to examine the structure of bovine class II gene products was made by Hoang-Xuan et al (1982b). These authors used anti-HLA class II product-monoclonal antibodies and anti H-2-class II product-antisera to identify antigens in bovine lymphocytes. They showed that such molecules are structurally similar to murine and human class II antigens. It remains to be shown that these are functional equivalents of the human and murine class II molecules and that they they are involved in the bovine MLC response. It would be valuable to attempt blocking of the MLC with the antisera which precipitate these molecules.

A monoclonal antibody has been described (Letesson et al, 1983) which detected a determinant on bovine B-cells but not T-cells; which is polymorphic, but probably supertypic in that it reacted with cells from 138 of 177 cattle. The molecule precipitated by this antibody was shown to be structurally similar to the murine and human class II antigens. This is confirmation of the report of

Hoang-Xuan et al (1982b) and the fact that the reagent used detected a polymorphic determinant strengthens the argument that there is a polymorphic bovine equivalent to the HLA-D region.

Thus it seems clear that there are genes in cattle which are linked to the BoLA-A locus and whose products control MLC response. There is also good evidence that there are antigens with a structure, tissue distribution and polymorphism similar to that of the class II products of other species. This is powerful evidence for a bovine class-II region but there has not yet been a serious attempt to define its products. It would be particularly important to attempt a correlation between the serologically detected B-cell antigens and MLC reactivity.

There have been reports of monoclonal antibodies (MoAbs) which appear to have BoLA specificity (Chardon et al, 1982; Spooner and Pinder, 1983). In no case have these reagents consistently shown the same specificity as a workshop cluster or even an alloantiserum. There are, however, no reports of systematic attempts to generate MoAbs for use as BoLA typing reagents, although Stone (1981) comments upon the desirability of such an approach. The recent HLA comparison test showed a major contribution by MoAbs to both class I and class II serology (Albert et al, 1984) and it would seem that the serological definition of BoLA has reached a stage where such tools could be of great value, particularly in the definition of class II products. Their major advantage over

alloantisera is that they are available in unlimited quantity.

Much work remains to be done on BoLA, but so far the only major difference between the bovine MHC and that of mouse and man, is the evidence that the bovine class I products may be under the control of a single locus. It seems highly likely that further class I loci will be revealed, but it is possible that they will be rather different from those of other species. Perhaps they are very closely linked to the BoLA-A locus or perhaps their products are much less antigenic than those of BoLA-A.

It is probably true to say that every laboratory working in the BoLA field believes that they have sera which detect the products of a second (or third) BoLA class I locus and some have published reports which appear to support this belief (Stear et al, 1982b). However, serology alone cannot provide sufficient information for the definition of a second locus. The identification of recombinants and the sequential precipitation of antigen molecules are two approaches which may be expected to yield such evidence.

It may be significant that the method of approach of the invesigators of BoLA has been rather different from that of their H-2 and HLA counterparts. This is partly due to the differences in the species; studies on mice lend themselves to intensive genetic manipulation by carefully controlled breeding programmes. This is

impossible in the human field although here records of ancestry and medical records are often very good, and human breeding is much more random than cattle breeding. The study of BoLA has been characterised by the preparation of antisera with extremely good specificity, made possible by the ability to collect large volumes of blood for serum and absorption and by the use of planned immunisations. BoLA workers have also made use of international comparison tests in the same way as the HLA groups. It is likely that, without either one of these, the literature would abound with reports of multiple BoLA loci.

The origin and characteristics of the cattle

The origins of the cattle of Africa

The classification and origin of the cattle of Africa has been the subject of a number of reviews, notably those by Epstein (1971) and Mason and Maule (1960). Both of these studies relied heavily on physiological and anatomical characteristics in an attempt to trace the development of the cattle populations. The biochemical evidence which could prove useful in such a study is very limited. Baker and Manwell (1980) reviewed the literature on the biochemical polymorphisms of cattle. The very scanty information largely supports the basic ideas of both Mason and Maule (1960) and of Epstein (1971).

European breeds are believed to be a result of successive migrations and specialised selection, based upon the longhorn type which derives from the wild ox *Bos primigenius*, with subsequent additions from the shorthorned *Brachyceros* type (Mason, 1976).

Further human selection, combined with natural selection for fitness in hot and dry country, produced an animal in Baluchistan and Mesopotamia which was derived from the *primigenius* type but which had a large cervico-thoracic hump and a long, hanging dewlap (Epstein, 1971). This is the Zebu. Zebus entered Africa in two waves, the first arrived between 1,000 years BC and 50 years AD. They interbred with the earlier longhorns to form the Sanga, which have small humps and long horns. The Sanga

moved into central and Southern Africa.

The second wave of Zebus have thoracic humps and began to enter Africa in the 4th century AD. The major importations, however, did not begin until the Arab invasions which began in 669 AD. This second wave of Zebus displaced the Sanga in large areas, although in places Sanga/Zebu crosses seem to have been formed which still survive. The Zebus moved into West Africa, where a number of intermediate types were formed by crosses with the local humpless cattle. The Fulani breeds seem to be of this Zebu/longhorn mixture.

The spread of the Zebu over Africa is attributed by Mason (1976) to a superior milk yield and to its adaptation to tropical and sub-tropical climates. He asserts that they are more resistant than European breeds to drought, parasite challenge and a low nutritional plane. The Sanga was finally eliminated from Kenya by the rinderpest epidemic of 1890, for it appears to be more susceptible than the Zebu. Humpless cattle now remain only in the cooler areas of Africa and in the tsetse belt of West Africa, where they appear to be more trypanotolerant than other types (Murray et al, 1984a; 1984b). The relationship between the two waves of Zebus, the humpless cattle of West Africa and the European breeds is thus not fully known. It may be that successive domestications of the wild ox followed by intensive selection separates all of these groups.

Recently, attempts have been made to exploit European cattle in Africa. These attempts have only been successful where intensive disease control techniques have been used. European cattle of the Bos taurus type are unable to survive in most of Africa without this type of management.

The present day cattle of Kenya

The cattle found in Kenya today are largely of Zebu type. The small East African Zebu is the commonest form (Mason and Maule, 1960). These are stocky, shorthorned cattle, rather variable in size and colour. There is much local variation. The cattle owned by the Maasai tribe for instance, are larger and heavier than most. Even within the Maasai cattle there is great variation, which is presumably due, at least in part, to effects of nutrition and disease.

The second major Zebu group in Kenya is the Boran. The Kenyan Boran is a rather highly selected animal used principally for beef production. It derives from cattle brought into Kenya by Somali traders and sold to European farmers, the Maasai and the Meru. The "improved Boran" is the result of intensive selection for beef production, and may contain some Bos taurus blood. The Boran appears to be generally more resistant to heat and drought than European breeds, but it is more susceptible to these factors and to some diseases than the small East African Zebu (Young and Dolan, personal communication).

Disease resistance characteristics of African cattle

There is a growing body of opinion that there are real differences in the specific disease resistance and in resistance to heat, drought and exercise stress between the different types of cattle.

For example, it has been established that Zebus are usually more resistant than European cattle to ticks, as judged by the numbers of female ticks engorging (Seifert, 1971; Wharton et al, 1973). It is also known that there is considerable heritable variation within a type (Seifert, 1971).

It is also clear that there is considerable variation between breeds in their trypanotolerance. N'Dama cattle have been shown to be more trypanotolerant than Zebus as have West African shorthorns (reviewed by Murray et al, 1984a and 1984b).

Frisch (1975) and Dodt (1977) showed that Bos taurus/Bos indicus crosses and pure Bos indicus animals suffer less often and less severely from infectious kerato-conjunctivitis than pure Bos taurus animals. Adams et al (1979) quote evidence that different breeds show different levels of resistance to brucellosis, babesiosis and tuberculosis.

Clearly, there are major differences between various breeds of cattle in a range of characteristics. The

differences between Bos indicus and Bos taurus are particularly striking.

The African environment

The environment faced by African cattle is likely to influence their performance and to exert a major selective pressure. The selective pressure exerted by the environment can be considered to have three major components; physical pressures due to climate, physical pressures due to management and exploitation, and disease pressures - which will be influenced by the first two.

The climate and vegetation

The climate and vegetation of East Africa is discussed in detail by Pratt and Gwynne (1977). It is characterised by large variations in temperature, rainfall, evaporation and vegetation. Rangeland, defined as "land carrying natural or semi-natural vegetation which provides a habitat suitable for herds of wild ungulates" (Pratt et al, 1966), represents 87% of Kenya's 569,260 square km. Much of Kenya's surface is defined by Pratt and Gwynne (1977) as eco-climatic zone V. This is land which is too dry for agriculture or forestry, it is mostly the territory of pastoral tribes and is sparsely occupied. In Kenya, large areas have been taken over for the use of wild animals. The vegetation is mostly thorn bush-land, Commiphora woodland and grassland and rainfall seldom exceeds evaporation. Most regions experience two peaks of rainfall, one in April and one in November. Day-time temperatures are consistently above 30 degrees

Centigrade and at night do not fall far below 20 degrees Centigrade.

In North Western Kenya, there is an area (about a quarter of Kenya's land area) which is classified as eco-climatic zone VI, semi-desert. This is a region of intense heat and desiccating conditions, annual rainfall seldom exceeds 300 mm while evaporation is frequently greater than 3,000 mm. The mean daily maximum temperature of this region is between 32 and 37 degrees Centigrade. The dry season is associated with hot, drying winds. This land is occupied solely by pastoral tribes which survive by "nomadism and expediency".

Apart from the small area of barren Afro-Alpine land (eco-climatic zone I) around Mount Kenya, the remaining quarter of Kenya's land is of eco-climatic zones II-IV. This is a much less demanding climate than that found in zones V and VI. Zone II is humid to dry sub-humid and is characterised by forest, grassland and bushland. Zone III is dry sub-humid to semi-arid which is not suitable for forestry but carries a vegetation of moist woodland, bushland or savanna. Large areas are under range-type cattle management; some areas are more intensively stocked and some are under crops.

Zone IV land is of marginal agricultural potential carrying dry forms of woodland and savanna. Some regions carry high densities of wild-life and it will generally carry cattle at about half the stocking rate of zone III

land, that is, at about 4 hectares per stock unit.

Traditional management of cattle

Cattle in East Africa fulfil a wide range of functions. This makes it very difficult to estimate their productivity in simple terms. The role of cattle in the traditional society is reviewed by Chavunduka (1976). He describes their spiritual role, their role as currency and their role as a source of traction. In addition to these functions they supply meat, milk and blood. Since most cattle are eventually eaten, beef production is part of their function, even when they are kept principally for milk (Mason, 1976).

As a result of these considerations, cattle have rarely been selected in the same way in Africa as they have in Europe. Many pastoralists would rather have a large herd of small cattle than a small herd of large cattle, regardless of their performance. This serves as a means of insurance against catastrophic loss due to drought or disease and is also influenced by the traditional regard of wealth in terms of cattle numbers. European selection is characterised by heavy culling of less productive individuals, while in Africa an animal is rarely culled while it can walk. On the other hand, there is some selection by selective castration of bulls and by allowing only selected bulls to mate. The selection is normally done by choosing those bulls which produce good calves or whose daughters are good milk producers. How

thoroughly and consistently this is done is hard to judge, but it will certainly play a part in governing the genetic make up of a herd.

Disease challenge

The disease challenge faced by African cattle is generally very much greater than that faced by European breeds. Little detailed information is available. However, it is clear that ticks, tick-born diseases and trypanosomiasis are major constraints on cattle productivity in East Africa (Irvin et al, 1981; Ristic and McIntyre, 1981; Murray et al, 1984a).

The object of the study

There is overwhelming evidence that the MHC plays a crucial role in the immune response of an individual. There is a great deal of work on the ruminant immune system presently being performed, much of it in African cattle because these animals are severely limited by a range of diseases (Morrison, 1985). Many aspects of this work require an ability to define BoLA products if they are to make further progress.

African cattle have been under intensive and highly localised disease challenge for many generations and they have generally not been selected strongly by man. Therefore, Africa and particularly East Africa with its wide range of cattle types and environments, appears to be an ideal place to begin a search for associations between the MHC and fitness for a given environment, both because such information would have its greatest value here and because associations are expected to be easily seen.

If MHC haplotypes could be identified which confer resistance (or which do not confer susceptibility) to a particular disease, they would be of enormous value in organising a breeding programme aimed at improving the productivity of African livestock. The large areas of East Africa which are unsuited to the production of crops and to intensive European-style cattle breeding are a resource which is presently under-used. Tourism is the only product of some areas while others are entirely

unproductive. Less arid land does carry cattle but their productivity is severely limited by environmental factors, particularly disease. An attempt to raise the productivity of this land by improving the performance of cattle under such conditions would be worthwhile and the MHC may well have a major part to play. Certainly it is the only known genetic marker with any hope of providing information on the performance of animals in the face of disease.

Preliminary work on the serology of Kenyan Bos indicus breeds using sera produced in European cattle, showed that these sera were inadequate for the definition of the MHC in African cattle. Therefore, before any attempt to investigate the role of the MHC in African cattle was possible, it was necessary to raise new antisera, investigate anew the reactivity of the existing ones and to make a large scale attempt to use these reagents to characterise the MHC of East African cattle.

Chapter 2

MATERIALS AND METHODS

Cattle

The cattle used in the study are described in table 2.1. The results of BoLA typing a total of 1,678 cattle are reported here. They were from a wide variety of herds in a wide variety of climatic regions. Figure 2.1 shows the areas from which the Kenyan cattle came.

Six hundred and sixty two Borans were BoLA typed. They were from one of two sources; 592 were from the Kapiti Plains Estate. This is a large ranch on the plains about 60 Km from Nairobi. The eco-climatic classification of this area according to Pratt and Gwynne (1977) is zone 4 - semi-arid. The remaining 70 Borans were from a group in the Trans Mara district of Western Kenya. All of the Borans were of the type described by Epstein (1971) as "improved". The Trans Mara Borans were all bought from Rumuruti in central Kenya. The origin of the Kapiti herd is not fully known, but it probably also came in large part from the Rumuruti area. The Kapiti herd is probably rather in-bred but is large, containing 1000 breeding females and approximately 40 active bulls.

A total of 606 animals of "Zebu type" were also BoLA typed. These were a much more mixed group than the Borans. Sixty two were from the Kafue flats in Zambia. They are certainly a representative sample from this area having been collected from a large number of different

herds. Another 148 Zebus were originally from the Orma tribes who live in the arid regions around the Tana River in Eastern Kenya. There is good evidence that these cattle, which live under a high trypanosome challenge, are more trypanotolerant than Borans (Ismail et al, 1985). Ninety Zebus were from the Galana Ranch in South Eastern Kenya. This is another arid region with a high trypanosome challenge. A total of 116 Zebus were from the Kaisut Desert in Northern Kenya. They were obtained from the local Samburu tribesmen and were from several different herds. This is a semi-desert region with low disease challenge but extremely hot and dry. Camels are tending to replace cattle as livestock for milk, meat and transport in this region. The remaining 190 Zebus were from the Trans Mara district of Kenya. They were Maasai owned and came from many different herds.

Forty nine Sahiwal/Friesian crosses were tested. These came from the Trans Mara district of Western Kenya. They were kept under ranch conditions.

Ten N'dama calves, brought to the International Laboratory for Research on Animal Diseases (ILRAD) from The Gambia as cryopreserved embryos, were also BoLA-typed.

In Edinburgh, 351 Bos taurus animals were tested. They were of a wide variety of breeds. One hundred and fifty eight came from the farms of the A.F.R.C. Animal Breeding Research Organisation in Scotland. The largest single breed of these was Friesian (92) but included were

representatives of 18 other breeds of cattle. From Norway 139 animals were tested, while the remaining 54 animals were from the English and Scottish Milk Marketing Boards (MMB and SMMB). They included eight Hereford bulls and 14 bulls of five other European breeds. This is an excellent representative sample of the highly selected cattle currently being used in Europe for milk and beef production under intensive management systems.

The classification and relationship of cattle is in some confusion. However, most authorities would classify all of the African cattle used in this study, with the exception of the N'damas, as being of Bos indicus type, and all of the European cattle as Bos taurus type (Epstein, 1971; Mason and Maule, 1960). This distinction is not sharp, and clearly they should not be considered to be the different species that these names imply, since they can and do interbreed. Certainly the Borans and Zebus form well defined races which are more closely related to each other than to the other groups. The small N'dama and Sahiwal cross populations are well separated from these and from the European cattle. The term Bos indicus is used in this study to refer to the Boran and Zebu cattle only, while the term Bos taurus refers to the European cattle.

Table 2.1 - The cattle

Type	Origin		Number
Improved Boran	Kapiti Plains	(Kenya)	592
Improved Boran	Rumuruti	(Kenya)	70
			662
Zebu	Kafue Flats	(Zambia)	62
Zebu (Orma)	Tana River	(Kenya)	148
Zebu	Galana Ranch	(Kenya)	90
Zebu (Samburu)	Kaisut Desert	(Kenya)	116
Zebu (Maasai)	Trans Mara	(Kenya)	190
			606
Sahiwal x Friesian	Trans Mara	(Kenya)	49
N'dama	The Gambia		10
Bos taurus	Scotland		158
Bos taurus	Norway		139
Bos taurus	SMMB & MMB		54
			351
			1678

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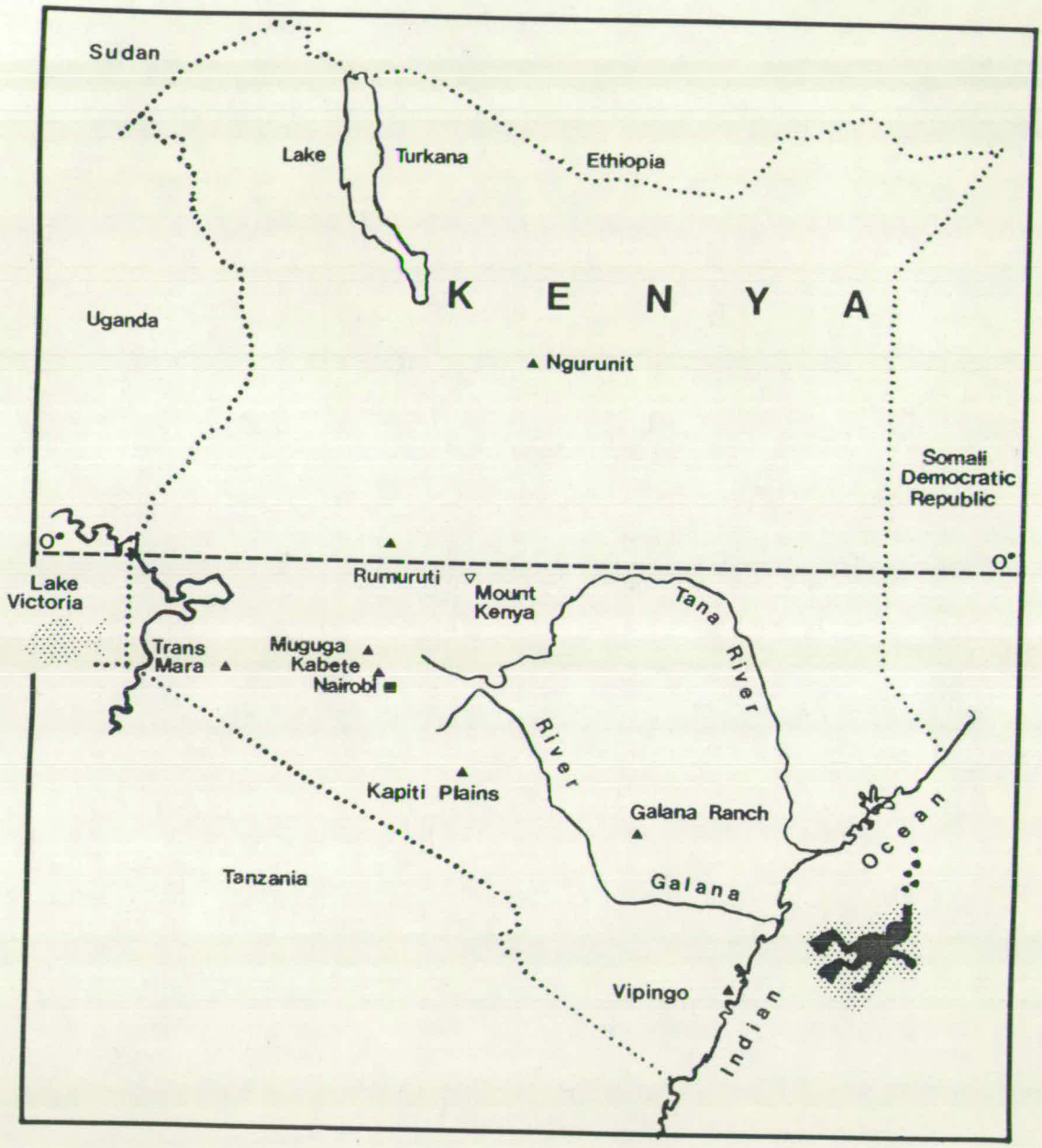


Fig 2.1 The origin of the cattle

Serum

Serum for use in the microcytotoxicity test was prepared as follows. Venous blood was collected from the jugular vein and allowed to stand overnight at room temperature. The serum was separated from the clot and centrifuged at approximately 1,500 x g for 35 minutes. The supernatant was collected and filtered through a five micron and then a 1.2 micron filter (Acrodisc, Gelman), and finally through a 0.45 micron filter (Millex). It was distributed into suitable aliquots and stored at minus 35'C or minus 60'C.

Immunisation

All of the antisera prepared in Kenya during the course of this study were made by alloimmunisation. The antisera used are listed in tables 2.2 to 2.4. The European sera were mostly prepared by skin grafting. Some were made by innoculation of peripheral blood leukocytes (PBL) while the rest were from parous cows. Most immunisations were made between dam/calf pairs (Spooner *et al*, 1979b).

The antisera raised in Kenya are listed in tables 2.3 and 2.4. They were made in two separate groups. Those with the prefix "KM" were either made at the Veterinary Research Department of the Kenya Agricultural Research Institute at Muguga, or at Vipingo Estates near Mombasa. The sera with the prefix "KK" were made at ILRAD using

Borans from the Kapiti Plains Estate.

The animals from the farm at Muguga were of extremely variable ancestry. The Bos indicus types would generally be considered to be of Zebu type. The Bos taurus types were either Friesian or Hereford, but may have had a small Bos indicus component. The immunisations performed at Vipingo were all between dam/calf pairs of "improved Boran". All were single immunisations by skin implant, the BoLA type of the animals involved was not known at the time of the implantation.

All primary immunisations (except those made by Theileria parva infected lymphoblast innoculation) were made by skin implant according to the method of Pringnitz et al (1982). Briefly, the immunisation procedure was as follows. Donor and recipient were lightly sedated with 2% Rompun (Bayer) given intramuscularly. The hair at the back of one of the donor's ears was shaved and the skin cleaned with soap and then alcohol. An area of skin at the base of the recipients neck was similarly prepared. Local anaesthetic (Lidothesin 1%, Pharmaceutical Manufacturing Co.) was infiltrated into these areas. An incision approximately two cm long was made through the skin in the recipient's neck. A pocket was made between the skin and the underlying muscle by probing with a scalpel handle. The loose skin at the back of the donor's ear was grasped with forceps and a piece of skin of approximately one square cm was removed. This skin was pushed into the pocket on the recipient's neck.

Streptomycin sulphate powder (Pharmaceutical Manufacturing Company) was dusted into the wounds which were then closed by one or two sutures.

Four typing sera resulted from immunisation on two occasions with Theileria parva infected cell lines. The BoLA-A locus phenotype of these lines was confirmed before use and they remained the same as those of the line donors.

The titre of the recipient serum in the standard microcytotoxicity test against the donor's lymphocytes was measured after about 14 days. If it was found to be sufficient (above 1:4), a quantity of up to one litre of blood (depending upon the size of the animal) was taken, otherwise the immunisation was repeated. Occasionally the second immunisation was by subcutaneous and intramuscular injection of PBL from approximately one litre of donor blood. PBL were collected and separated in the same way as PBL for absorption, except that the final resuspension was in five ml of RPMI 1640 (Gibco).

Titres were found to be extremely variable following first immunisation. There was no obvious relationship between the BoLA compatibility of the donor and the recipient, and the titre of serum. A second immunisation always produced a serum with a titre of 1:32 or above. Table 2.5 shows the titres of sera following the 20 immunisations performed at ILRAD of which six were boosted. It is not possible to draw conclusions about the

relationship between BoLA-type and response, as the donors selected usually had at least one undetected A locus allele and since the recipient was invariably the donor's calf the degree of matching could vary from complete matching of two MHC haplotypes (when the animals were inbred) to complete mismatching at all loci, without being distinguishable by BoLA-typing.

The number of animals with which a serum reacted was always much more limited following a single immunisation than following two. Several sera are used as typing reagents with no absorption (see tables 2.3 and 2.4), and these all result from a single immunisation. A second immunisation often produced a "pan-reactive" serum which defied all attempts to render it operationally monospecific. For this reason second immunisations were avoided as much as possible.

Table 2.2 - The Edinburgh sera

Serum	Number of absptns *	Lab performing immunisation !	Working dilution	Specificities detected
EDA001	2	Edinburgh	1:4	w1 & w3
EDA002	2	Edinburgh	1:4	w2
EDA005	2	Edinburgh	1:4	w13
EDA007	3	Edinburgh	1:4	w5
EDA008	0	Edinburgh	1:4	w1
EDA009	1	Edinburgh	1:8	w6
EDA010	1	Edinburgh	1:4	w6
EDA011	2	Edinburgh	1:8	w7
EDA012	1	Edinburgh	1:4	?
EDA013	0	Edinburgh	1:8	w8
EDA014	0	Edinburgh	1:4	w9
EDA016	1	Paris	1:8	w13
EDA017	1	Paris	1:4	w7
EDA028	4	Paris	1:16	?
EDA030	1	Paris	1:4	?
EDA038	2	Paris	1:8	w16
EDA039	1	Paris	1:8	w3
EDA040	1	Paris	1:16	EU12
EDA043	2	Paris	1:8	w16
EDA047	4	Paris	1:4	w12
EDA062	2	Edinburgh	1:16	w2
EDA063	5	Edinburgh	1:16	w8
EDA064	2	Edinburgh	1:4	w2
EDA065	1	Edinburgh	1:8	w2
EDA066	4	Edinburgh	1:4	w7
EDA068	3	Edinburgh	1:8	w7
EDA069	5	Edinburgh	1:4	w10
EDA071	2	Edinburgh	1:8	w10
EDA072	1	Edinburgh	1:4	?
EDA073	2	Edinburgh	1:16	w11
EDA074	1	Edinburgh	1:16	?
EDA075	1	Edinburgh	1:8	w6
EDA076	1	Edinburgh	1:8	w11
EDA077	0	Edinburgh	1:8	?
EDA078	2	Edinburgh	1:4	w20
EDA080	4	Edinburgh	1:4	w8
EDA081	1	Paris	1:8	?
EDA082	1	Paris	1:8	w4
EDA083	4	Edinburgh	1:4	w1
EDA085	3	Edinburgh	1:4	?
EDA086	2	Edinburgh	1:4	?
EDA092	1	Edinburgh	1:4	?
EDA093	2	Edinburgh	1:4	?
EDA094	3	Edinburgh	1:8	?
EDA096	2	Edinburgh	1:16	w10
EDA097	2	Edinburgh	1:32	w6
EDA098	3	Edinburgh	1:4	?
EDA099	2	Edinburgh	1:4	?

.....continued

Table 2.2 - continued

Serum	Number of absptns *	Lab performing immunisation !	Working dilution	Specificities detected
EDA100	0	Berne	1:16	?
EDA101	4	Edinburgh	1:16	?
EDA102	4	Edinburgh	1:8	?
EDA104	3	Edinburgh	1:4	?
EDA105	2	Edinburgh	1:4	?
EDA107	0	Berne	1:8	?
EDA109	5	Edinburgh	1:16	?
EDA110	3	Berne	1:4	w11
EDA111	4	Berne	1:8	?
EDA112	3	Edinburgh	1:4	?
EDA113	1	Edinburgh	1:4	?
EDA114	3	Paris	1:8	?
EDA115	2	Edinburgh	1:8	w2
EDA116	1	Edinburgh	1:4	?
EDA117	2	Edinburgh	1:4	?
EDA118	3	Edinburgh	1:8	?
EDA120	4	Edinburgh	1:4	?
EDA121	2	Copenhagen	1:8	?
EDA123	4	Berne	1:32	?
EDA124	4	Paris	1:4	?
EDA125	3	Edinburgh	1:4	?
EDA126	5	Paris	1:4	?
EDA127	4	Edinburgh	1:4	?
EDA128	4	Paris	1:4	?

* Number of donors of PBL used in absorption.

! Spooner et al, 1979a; Anon, 1982; INRA, 1982.

Table 2.3 - The Kenya Muguga Sera

Serum		Donor tissue	Recipient	Absptns	Diltn	Spcfcty
KMA001		(x) w7/-	(t) w7/w9	0	1:4	?
KMA002		(t) w7/w9	(X) w7/-	2	1:32	?
KMA003		(i) w4/w10	(i) -/-	3	1:4	w10
KMA004		(i) -/-	(i) -/-	2	1:8	KM4
KMA005	*	(t) ?	(t) ?	1	1:4	?
KMA006	*	(t) ?	(t) ?	2	1:4	?
KMA007	V	(i) ?	(i) -/-	1	1:4	w7
KMA008	V	(i) -/-	(i) ?	0	1:4	?
KMA009		(i) -/-	(i) ?	0	1:4	?
KMA010		(i) w8/-	(i) w8/-	1	1:16	KM18
KMA011		(i) W11/-	(i) -/-	2	1:4	?
KMA012		(i) W8/-	(i) w8/-	2	1:4	?
KMA014		(i) -/-	(i) -/-	2	1:8	KK1
KMA015	*	(t) ?	(t) ?	1	1:4	?
KMA017		(i) -/-	(i) W11/-	1	1:4	KM17
KMA018	V	(i) -/-	(i) ?	1	1:16	KM18
KMA021		(i) W11/-	(i) -/-	2	1:4	?
KMA022	!£	(t) w6/w8	(t) w6/w10	3	1:16	KM22
KMA023		(i) -/-	(i) ?	1	1:8	?
KMA024		(i) -/-	(i) w4/w10	4	1:16	KM4
KMA025	!£	(t) w6/w8	(t) w6/w8+	3	1:8	?
KMA026	*	(t) ?	(t) ?	1	1:4	?
KMA027		(i) w10/-	(i) -/-	1	1:8	?
KMA028		(i) -/-	(i) w10/-	2	1:16	?
KMA029		(t) -/-	(i) -/-	3	1:8	KM17
KMA030		(i) -/-	(i) -/-	2	1:8	?
KMA031		(i) -/-	(i) -/-	3	1:8	?
KMA032		(i) -/-	(i) w9/-	4	1:4	?
KMA033		(i) w9/EU12	(t) w9/-	3	1:16	KM33
KMA034	!£	(t) w11/w20	(t) w11/w20	4	1:2	?
KMA035		new batch of KMA005				
KMA036		new batch of KMA026				
KMA037		(i) w8+/-	(x) -/-	3	1:8	?
KMA038	V	(i) ?	(i) -/-	3	1:16	w6
KMA039		(i) -/-	(i) -/-	3	1:4	?
KMA040		(i) w8/-	(i) -/-	5	1:8	?

(i) *Bos indicus* type
(t) *Bos taurus* type
(x) Cross-bred

* An absorption performed in Kenya upon a serum raised in Edinburgh.
! Immunisation with *Theileria parva*-infected lymphoblasts. Except for ! immunisation was by implant, sometimes boosted with PBL.
£ Except for £ immunisation was between dam/calf pairs.
V Immunisation performed at Vipingo using Borans. Others were performed at Muguga.

Table 2.4 - The Kenya Kabete Sera

Serum	Donor tissue	Recipient	Absptns	Working Diltn	Spcfcty
KKA001	(i) -/-;KMA014	(i) -/-	0	1:4	KK1
KKA002	(i) -/-;KMA014	(i) -/-	0	1:16	KK1
KKA003	(i) -/-;EDA118	(i) -/-	0	1:4	KK3
KKA004 £	(i) w10/KM18	(i) KM18/-	2	1:16	KK4
KKA005	(i) w6/-	(i) w6/-	2	1:16	?
KKA006	(i) -/-;KMA014	(i) w6/-	1	1:8	?
KKA007	(i) -/-	(i) w10/-	1	1:4	?
KKA008	(i) w1/-	(i) w4/-	1	1:4	?

(i) *Bos indicus* type
All immunisation was by skin implant, sometimes repeated.

Except for £ immunisation was between dam/calf pairs

Table 2.5 - Maximum dilution of sera giving at least 40% killing of donor lymphocytes in the microcytotoxicity test.

	No of sera	
Titre	1 immunisation	2 immunisations
<1:2	5	0
1:2	2	0
1:4	0	0
1:8	7	0
1:16	0	0
1:32	1	0
>1:32	5	6
	20	6

Absorption of sera

Before a serum could be considered to be operationally monospecific, and therefore a useful test reagent, it was absorbed with PBL from reacting animals. If this process removed activity for all animals then the serum could be assumed to have activity for the same antigen on all of these animals. The method used varied considerably depending upon the volume of serum being absorbed. Following absorption, the serum was always tested for cytotoxicity against cells from the animal which provided PBL for the absorption, to confirm that all activity for these cells had been removed.

Cells for absorption were prepared as follows. Blood was collected into an equal volume of Alsever's solution (Gibco), and buffy coat cells were then collected following centrifugation for up to 40 minutes at 1200 x g. The erythrocytes were removed by a flash lysis with a large volume of distilled water, followed by sufficient Hank's balanced salt solution (HBSS, Gibco) at 10 times working concentration to restore isotonicity. The lymphocytes were pelleted by centrifugation and the supernatant was poured off. Cells were resuspended in fresh HBSS and centrifuged again. Then the supernatant was poured off and the cells were re-suspended in the serum to be absorbed. In most cases, a ratio of one volume of packed cells to three volumes of serum was sufficient to remove activity for the cells. The cells and serum were incubated at room temperature for 40

minutes with continuous gentle mixing. Then the cells were removed by repeated centrifugation followed by filtration as for the sera above.

Monoclonal Antibodies

Three mouse monoclonal antibodies (MoAbs) were used as typing reagents. They were made at ILRAD by I. Morrison, A. Teale and P. Lalor. The immunisations which lead to them are described in table 2.6. They were used in the form of mouse ascitic fluid (MA 4.1 and P3) or as tissue culture supernatant (MA 7.1).

The extremely high titre in the cytotoxicity test of the ascites, greater than 1:100,000 in one case, created problems. The small amount of fluid carried between test wells by the dispensers was not significant with alloantisera which have titres of less than 1:300, but with monoclonal antibodies in the form of mouse ascites could cause cell death in the adjacent wells. This was avoided by, where possible, using care not to allow dispensers to touch the drop of reagent and by rinsing dispensers in 5M sodium hydroxide after they came in contact with such reagents. The absence of carry-over was ensured by placing negative control sera in the well following that containing a MoAb, if this well was not completely negative then carry-over was assumed to have occurred and the following wells were scored as untested until a negative was encountered. In practice such a situation was rarely encountered. Problems associated

with this carry-over of monoclonal antibodies have also been reported in HLA-typing (Bodmer et al 1984).

The MoAb (P8) was used to examine macrophage numbers in PBL preparations. This was prepared at ILRAD by P. Lalor and is specific for monocytes (Lalor et al, 1985). It was used as mouse ascites at a dilution of 1:400.

Table 2.6 - The ILRAD Anti-MHC Monoclonal Antibodies

Antibody	Ig Class	Immunising tissue	Specificity	Working dilution
MA 4.1 (Ascites)	IgG1	(i) PBM w10/KM18 *	KK4	1:10,000
MA 7.1 (TC Sup)	IgG2A	(t) PBM w7/w10 !	W10 & KM17	1:10
MA P3 (Ascites)	IgG2A	(i) PBM KM18/-	KM18,w2	1:1,000

(i) Bos indicus
(t) Bos taurus

(TC Sup) Medium from an overgrown culture.

(Ascites) Dialysed ascitic fluid from mice innoculated with hybridoma cells.

* KMA003 & KKA004 positive
! KMA003 & KKA004 negative

Cell Lines

Bovine lymphoblastoid cell lines, infected with *Theileria parva* and used for some immunisations, were provided by other groups at Muguga. They were established and maintained according to Stagg *et al* (1981), and were grown in RPMI 1640 with 10% foetal bovine serum (FBS, Gibco). Before use they were BoLA-typed in the standard lymphocytotoxicity test using a low toxicity complement source.

Complement

The source of complement for the BoLA-typing of PBL was commercial freeze dried rabbit serum (Cedar-Lane, Low-Tox-H) Complement was pre-screened for cytotoxicity and complement activity. It was stored at minus 20'C, and was reconstituted with distilled water, mixed gently and placed on ice. Reconstituted complement was never re-frozen or used after more than one hour.

Lymphoblastoid cell lines were typed using a different complement source since they were found to be susceptible to lysis by the commercial complement. The complement used for this was prepared from pooled rabbit serum obtained from the ILRAD colony. It was absorbed on ice for one hour with bovine PBL collected as for serum absorption. Approximately one volume of packed cells was used to absorb three volumes of serum. It was then screened for cytotoxicity and complement activity against both cell lines and PBL.

The lymphocytotoxicity test.

All of the BoLA typing was performed using a standard procedure. The test used was a modification of that originally developed by Gorer and O'Gorman (1956) and is described by Teale *et al* (1983).

Where possible, lymphocytes were separated and tested within 24 hours, with 48 hours being the maximum time between collection and testing of unpreserved samples. Alternatively, the blood was cryopreserved. The cryopreservation process is described in chapter 3.

Lymphocyte preparation from heparinised samples

Venous blood (either jugular or caudal) was collected into sterile vacutainers containing lithium heparin (Becton-Dickinson). Lymphocytes were separated from fresh blood as follows. 2ml of whole blood was layered onto 3ml of a mixture of Ficoll 400 (5.7% w/v) and sodium diatrizoate (9.0% w/v) (s.g.1.077, Ficoll-Paque, Pharmacia). The tubes were centrifuged at 1200 x g for 20 minutes at approximately 15'C. The lymphocyte-rich interface between the plasma and the Ficoll-Paque was drawn off with a pasteur pipette and mixed with approximately 10 ml of HBSS in a 10ml polystyrene conical centrifuge tube (Sterilin). The tubes were then centrifuged at 130 x g for 5 minutes, at 15'C. The supernatant was poured off and 2ml of distilled water was added to the cell pellet while it was being mixed

vigorously. After approximately five seconds of mixing, 2ml double strength HBSS was added and mixing was continued for another five seconds. A further six ml of HBSS was then added to the tubes and they were again centrifuged at 130 x g for 5 minutes. The supernatant was poured off and the cell pellet re-suspended, then 10ml of HBSS was added and the centrifugation repeated. The pellet was then re-suspended in 1ml 10% FBS in HBSS (test medium). The cells were counted in a Neubauer chamber under phase contrast to allow an estimate of viability, and adjusted to 2.5×10^6 /ml in test medium. Only samples in which 80% or more of lymphocytes were viable were considered acceptable for testing.

The resultant cell suspension was completely free of erythrocytes as a result of the flash lysis. Platelet contamination was variable, and when it was considered excessive the cells were centrifuged at 100 x g for 5 minutes and the platelet-rich supernatant was discarded. Granulocyte contamination was rarely over 5%.

Lymphocyte preparation from defibrinated blood

Defibrination of blood samples provided a cell preparation completely free of platelets and with very low granulocyte numbers, but it was not always practical under field conditions, particularly when cattle were sampled in small and scattered groups.

Defibrination was performed by collecting

approximately 6ml of venous blood into a 10ml vacutainer (Venoject) containing approximately 2ml of small glass beads. The vacutainers were immediately placed on a rotary mixer and after 10 to 15 minutes of mixing a fibrin clot formed around the beads. Following defibrination, lymphocytes were separated in the same way as for heparinised blood, except that it was found that only one wash was required since there were negligible numbers of platelets in the preparation.

The absence of macrophages in PBL prepared from defibrinated blood was demonstrated by the use of the monoclonal antibody P8 (specific for macrophages) in an indirect immunofluorescence test. PBL were incubated with P8 ascites at 1:400 in RPMI 1640 with 0.1% sodium azide and 1% FBS (FACS medium) at four degrees Centigrade for 30 minutes. They were washed twice in cold FACS medium and incubated at the same temperature for 30 minutes with a 1:10 dilution in FACS medium of a Fluorescein isothiocyanate-conjugated rabbit-anti-mouse immunoglobulin serum prepared by staff at ILRAD according to the method of Brandtzaeg (1973). The cells were again washed twice in FACS medium and their fluorescence was examined on a FACS II cell-sorter (Herzenberg and Herzenberg, 1979). Figure 2.2 is a plot of cell numbers versus fluorescence following this analysis. It shows negligible macrophage numbers in a defibrinated sample, and considerable numbers in heparinised PBL collected at the same time from the same animal.

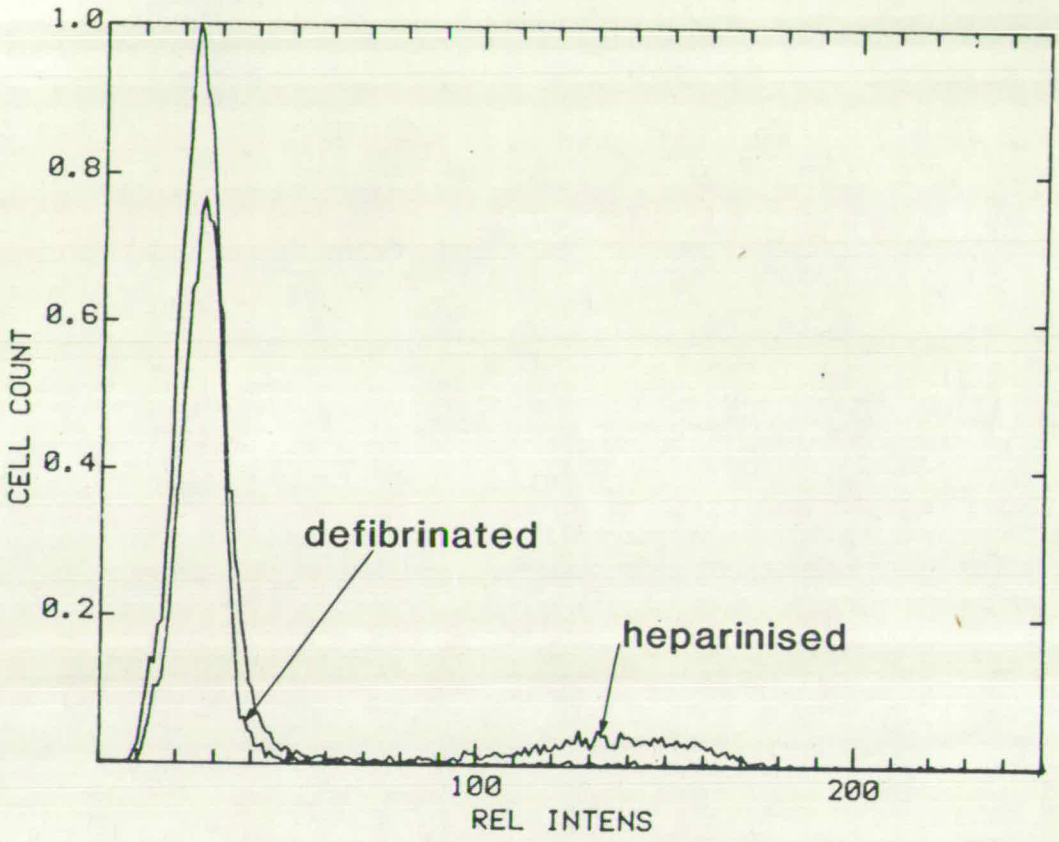


Fig 2.2

FACS profile following incubation of heparinised and defibrinated PBL with a MoAb specific for macrophages

Microcytotoxicity testing

The microcytotoxicty test, which was the basis of the BoLA typing was performed as follows. Terasaki plates (Nunc) were flooded with liquid paraffin, allowed to stand for ten minutes or more to allow air bubbles to leave and then all excess liquid was poured off. This left the wells completely full but without liquid paraffin between wells.

The typing antisera were diluted in test medium to their appropriate working dilution in Terasaki monoclonal cluster plates (Costar). Then one micro litre was added to each well of the Terasaki plates using a Multi dispenser (Hamilton). The sera were arranged so that each plate carried six different reagents, one in each column. This was found to be superior to the more common "multi-plate" arrangement (in which each plate carries a different reagent in each well), in a number of respects. It meant that the typing sera needed to be held at room temperature for short periods of time, and it minimised the chance of carry over as cells and complement were distributed. It had the disadvantage that it was wasteful of materials to test animals in other than groups of ten. However, this was not a major drawback.

Following the distribution of antisera, the plates were stored at minus 60'C until required. They were never kept for more than two months before use.

After the plates had reached room temperature, one

micro litre of cell suspension, prepared from heparinised or defibrinated blood as described above, was distributed into each well (2,500 cells per well) using a multi dispenser. Then one micro litre of complement, diluted 50% in test medium was added and the plates were incubated at 37'C for 80 minutes.

After incubation, one micro litre of 1.25% Eosin Y (Fisher) in HBSS was added to each well. After three to four minutes, two micro litres of 40% formaldehyde (Kobian Ltd) buffered to approximately pH 7 with 0.15M di-sodium hydrogen phosphate (Sigma) was added. The plates were then stored over-night at four degrees C before being read.

Reading was performed under phase contrast microscopy using a Nikon microscope at a total magnification of 150x. Dead cells were identified by their failure to exclude eosin. Scoring of the wells was as follows:

Not tested	.
0-10% of cells dead	-
11-20% of cells dead	2
21-40% of cells dead	4
41-80% of cells dead	6
81-100% of cells dead	8

Scores of - and 2 were considered negative and scores of 6 or 8 were considered positive. The partial killing scored as 4 was not often seen and was normally considered negative as a result of the analysis performed at the two BoLA comparison tests (Spooner et al, 1979a; Anon, 1982).

Chapter 3

THE DEVELOPMENT OF A MEANS OF CRYOPRESERVING LYMPHOCYTES IN WHOLE BLOOD

Introduction

Following the collection of blood, it was found to be necessary to separate and test the lymphocytes within 24 hours in order to reliably obtain cells of sufficient viability to be of use in the cytotoxicity test. This placed severe constraints upon the collection of samples from areas more than a short distance from the laboratory.

The road conditions in Kenya are such that a journey of 150 miles can take eight hours. During this time the samples are violently shaken and exposed to temperatures of up to 40'C in the shade. Direct sunlight very rapidly raises the temperature of a sample of blood sufficiently to kill every cell. It was found to be essential to minimise temperature fluctuations by storing the samples in an insulated box.

Even with such precautions, the fact that a maximum of 50 samples could be tested in one day meant that to collect a useful number of samples from a given area required a series of expensive and time consuming journeys, many of which yielded 40 or less samples with a viability adequate for BoLA-typing.

In an attempt to overcome this problem, a brief investigation of means of cryopreserving lymphocytes in the field was made. It was not an attempt to exhaustively

examine the effects of various parameters on the success of cryopreservation, it simply used an empirical approach to find a system which worked under the unusual conditions facing this study. In this it succeeded, and the work is reported by Kemp and Teale (1984).

The requirements of such a technique were; that it should be fast and simple to perform, so that many samples could be processed rapidly; that it should require no electricity supply; and that it should require no equipment that could not be carried in a Land Rover.

Since no means of separating lymphocytes was available in the field, it was necessary to freeze whole blood. Because the conditions required for the successful cryopreservation of lymphocytes destroy other cells (Mazur, 1976), this would certainly contaminate the sample with cell debris. It is likely that much of this debris is potentially cytotoxic, but it was hoped that by rapid freezing and then rapid dilution following thawing, that the cell loss due to this effect could be minimised.

Many "recipes" exist which describe conditions for cryopreserving a wide variety of cell types (for a review see Mazur, 1976). Most techniques require the controlled cooling of separated cells, in the case of lymphocytes, this is generally at a rate of about 1°C/minute. Tate and Cram (1982) described a method for the cryopreservation of bovine lymphocytes in whole blood by such a method.

An alternative to a controlled rate cooling technique is a two-step cooling such as that described by Knight et al (1977). Their method for the cryopreservation of human lymphocytes requires that samples of whole blood be held at -10'C for a maximum of two minutes, but this precise timing would not be feasible in the field. A two-step freeze has also been described for sheep lymphocytes by Stear et al (1982a) and was modified for cattle lymphocytes by Oliver et al (1985).

Materials and Methods

Initial collection and treatment of blood samples

Venous or caudal blood was collected into vacutainers containing lithium heparin (Becton-Dickinson). Two ml was transferred to a 5ml polystyrene freezing vial (Nunc) which was placed into a bath of iced water. After five to ten minutes, two ml of ice-cold dimethyl sulphoxide (DMSO, Sigma) at an appropriate concentration in HBSS, was added dropwise. The samples were mixed by inverting twice and immediately transferred to the next stage of the freezing process.

Controlled-rate freezing

The cooling of samples at a controlled rate was achieved by the use of boxes made of expanded polystyrene of a variety of wall thicknesses. Samples were packed into these, and then placed in a closed container of solid carbon dioxide. The temperature inside the box was monitored by means of a thermo-couple which was inserted

into one of the samples.

Methanol bath

A bath, well insulated with expanded polystyrene and containing approximately four litres of 50% methanol and 50% water, was used for the first stage of a two-step freezing. The methanol/water was pre-cooled in a box of solid carbon dioxide, and its temperature maintained during the freezing process by the addition of small pieces of carbon dioxide, which also served to vigorously mix the liquid in the bath.

Thawing and separation

After freezing, the lymphocytes were separated as follows. The samples were rapidly thawed in a water bath held at 37'C, and then transferred to a 15ml conical centrifuge tube containing 2mls HBSS with 25i.u./ml heparin (Novo). After mixing, a further 4ml heparinised HBSS was slowly added with mixing. The tubes were then centrifuged at 210 x g for 5 minutes. All but 1ml of the supernate was discarded and the cells resuspended. 10mls of heparinised HBSS was then added and the tubes were centrifuged at 150 x g for 5 minutes. The supernatant was poured off and the cells resuspended in 1ml HBSS. This suspension was layered on to Ficoll-Paque, and the lymphocytes separated and washed as described above for heparinised samples (Chapter 2; "Lymphocyte preparation from heparinised samples").

Results

With a final DMSO concentration of 10% the controlled freezing technique gave cell preparations which did not have a consistently acceptable viability. The explanation of the variability of this method may have been that the cooling rate was extremely variable. Samples at the edge of the box cooled much faster than those in the middle, and even when all samples were placed at the edge, the rate of cooling varied with the total number of tubes in the box. It was found that for a sample to reach a temperature of minus 40 'C could take over two hours, even when the polystyrene box was wafer thin.

The two-step freezing technique, using a final DMSO concentration of 10% and a methanol temperature of minus 25'C gave cell preparations with consistent but unsatisfactory viabilities, about 75%.

The viability of the cells was relatively insensitive to variations in the temperature of the methanol bath. Varying this between -25'C and -40'C showed a slight improvement at the lower temperatures. The mean viability at -35'C was approximately 80%.

Next, the DMSO concentration was varied using a methanol bath at minus 35'C. A dramatic increase in cell viability was seen when the concentration of DMSO was raised to 15%. At concentrations higher than this, most cells maintained a good viability, but some samples showed

significantly lowered numbers of viable cells. This presumably was the result of damage to the cells caused by the DMSO during the thawing process. Table 3.1 shows the result of freezing cells using these conditions.

It was shown that the viability of cells was unchanged if they remained in the methanol bath under these conditions for up to two hours.

Table 3.1: The results of freezing cells by the two-step method, using a methanol bath at -35'C and a final DMSO concentration of 15%.

Animal	% recovered *	% viable
1	34	89
2	52	90
3	19	90
4	68	87
5	30	92
6	46	89
7	23	83

* By comparison with cells prepared from two ml of fresh blood in conventional way described in Chapter 2.

Discussion

The two-step freezing method, using a methanol bath held at minus 35'C, and a final DMSO concentration of 15%, reliably gave cell populations with viabilities over 85%, which is more than adequate for BoLA-typing. Over three hundred cell samples were cryopreserved by this method and subsequently BoLA-typed, and the performance of the typing sera was unchanged with no evidence of an increase in background cell death.

The slow, dropwise addition of HBSS to the sample after thawing was found to improve viability, presumably by reducing the osmotic damage as DMSO left the cells. This was a compromise between the desirability of first rapidly removing toxins resulting from cell death during freezing, and also of rapidly diluting out the DMSO which is itself toxic at room temperature, and of reducing the damage caused by rapid dilution. It seems likely that slow dilution following thawing becomes more critical as the DMSO concentration used in the freezing process is increased.

The recovered cell preparation was completely free of platelets and erythrocytes. Macrophage numbers appeared unchanged by the freezing process. The destruction of the platelets may be significant in that they will release potentially toxic material into the preparation. For this reason defibrinated blood rather than heparinised blood might give even better results.

Chapter 4

STATISTICS AND COMPUTING

Introduction

There are two major statistical problems facing a study such as this. One is to define the tools of the analysis - the sera - and the second is to use these tools to analyse the distribution of the BoLA alleles. The first of these is a classical statistical problem which can be resolved, within limitations, by cluster analysis. Because of the complexity of the set of antigens which generate the antibody response, it is virtually unknown for two alloantisera to have identical reaction patterns. Even in the highly unlikely case of antisera which have only one population of cytotoxic antibodies directed against the same MHC antigen molecule, differences in the pattern of cross-reactivity would be expected. Therefore a study such as this generates a complex mass of results for each serum and it is almost never possible to immediately identify sera which are identifying the same antigens by a simple examination of the results. It is important that an analysis of the relationship between sera is not simply performed once, but that cluster analysis is used throughout the development of the reagents to suggest improvements that may be made by absorption or by further immunisation.

Cluster Analysis

The subject of cluster analysis is reviewed by Everitt (1974). There are two stages in any clustering process. The first is the establishment of a measure of similarity or dis-similarity between individuals (in this case the individuals are the sera), and the second is the process of merging individuals into clusters on the basis of these measurements.

The measurement of similarity

The observations made in this study were considered to be of the binary type, that is, the reaction of an animal's PBL with a serum could take only one of two values - positive or negative. For a large number of observations on two antisera (designated s1 and s2) there are four possible outcomes and these are represented by a, b, c and d in the 2x2 contingency table:

		s2 +	s2 −
s1	+	a	b
	−	c	d

In the case of cluster analysis of antisera the value "a" will represent absolute numbers of animals whose PBL react with both serum s1 and serum s2; "b" will represent

numbers which react with s1 but not s2; "c" will represent numbers reacting with s2 but not s1 and; "d" those reacting with neither serum. Thus there are two types of result, matched results (a and d) and non-matched results (b and c). Two highly similar antisera will generate large values in the matched categories and relatively low values in the non-matched categories. However, because most serum/PBL reactions give a negative result, even two very dis-similar antisera will have a large number of matched negatives (value c) so that this figure is of little value in determining similarity between antisera.

It is assumed that highly similar sera are detecting the same or closely related antigens, although sera with identical reaction patterns may of course detect entirely different epitopes on a given antigen molecule. The term "antigen" used here refers to a structure which probably has several antigenic sites (Lopez de Castro et al, 1984) and a cluster of sera which detect the same antigen may well detect different sites.

The following similarity coefficients have been used by various authors (reviewed by Everitt, 1974):

(i) $(a+d)/(a+b+c+d)$

(ii) $a/(a+b+c)$

(iii) $2a/(2a+b+c)$

(iv) $(2(a+d))/(2(a+d)+b+c)$

(v) $a/(a+2(b+c))$

(vi) $a/(a+b+c+d)$

They all give different weighting to different conditions. For the purpose of the clustering of antisera it is appropriate to give more weight to matched pairs than to unmatched pairs as is done by (iii) and (iv) above. The difference between these two formulae is that (iv) gives the same weight to matched negatives (d) as to matched positives (a) whereas (iii) gives no weight to matched negatives but increased weight to matched positives. When considering the extremely sparse reactivity of many typing reagents where more than 95% of observations may be negative, weighting matched negatives as is done by (iv) would not be reasonable since the large majority of matched negatives will be due to chance, and the converse of this is the justification for weighting matched positives more heavily than unmatched observations.

This suggests that the formula given in (iii) above is the best measure of similarity for clustering antisera and it is the formula chosen by Currie-Cohen et al (1980) in their analysis of BoLA-typing reagents. It has the

advantages of simplicity and intuitive appeal. It is superior to the correlation coefficient which is reported by many workers in the HLA and other MHC fields (de Weck et al, 1976 and Pickbourne et al, 1978). This is because the data generated by testing antisera against cells are very sparse and not suited to the treatment given by this test, which gives excessive weight to matched negatives and insufficient to matched positives.

However, there can be no absolute rule as to which method of calculating similarity is the best for this purpose, because as Sokal and Sneath (1963) say, each set of data must be considered on its merits. A similarity index based on (iii) above was the measure used throughout this study. For convenience it was expressed as a percentage so that it was calculated by:

$$\% \text{ SIMTY} = 100(2a/(2a+b+c))$$

Clustering

Having established a technique for measuring similarity it was necessary to decide how to merge antisera to form clusters. Everitt (1974) suggests that the so-called hierarchical clustering techniques are the most suitable to biological observations. These methods work by simply taking a set of distances or similarities between individuals and repeatedly merging those with the greatest similarity. The reverse procedure may also be used so that all individuals are considered to be part of

one cluster which is then progressively split.

The crucial problem of the hierarchical techniques appears to be the way in which they handle the emerging clusters. The distance between a cluster and another observation may be taken as the maximum of the distances between each member of the cluster and the other observation, or some form of average value may be determined for the cluster.

However, when clustering antisera the underlying hypothesis is that there is not a complete gradation of types of reagents, but that if the sera were perfectly monospecific the components of the clusters would coincide exactly. Thus, when two antisera are clustered together because they appear to detect the same antigen, it is invariably true that the most of the differences between the sera are not due to differences in the principle antibody population, but rather to the presence of extra antibodies (this is demonstrated by absorption studies of clusters, Spooner et al, 1980). If a serum has specificity for an antigen detected by a cluster as well as for another antigen, it would not be unreasonable to incorporate it into the cluster, but it would clearly be wrong to then "shift the location" of the cluster towards another cluster which detected the same specificity as the second antibody population in this serum. This is exactly what is done in conventional clustering when the distance between two clusters is reduced as a result of incorporating an intermediate individual into one of the

clusters. Such chaining of clusters could result in meaningless results. In this study for example, w7 and w10 in the Boran population would have been merged by such techniques. Cluster analysis of typing sera is not therefore an attempt to measure the degree of association of antisera but an attempt to identify the areas of overlap which are due to single antigens.

The obvious solution to this problem is to use a technique which does not seem to have been formally described in the literature. When two sera are merged to form a cluster, the behaviour of that cluster may be characterised by considering those animals which are positive for both to be positive for the cluster, and all others negative. In this way the sera within the cluster may be collectively represented by a single "phantom serum". Further comparisons may then be made between the cluster represented by this phantom serum and other sera.

This technique is more taxing in terms of the number of calculations to be made than are the conventional techniques because it requires that, after each merging of antisera or clusters, a new set of similarity measurements be made which incorporate the new cluster and treat it as if it were a new serum.

The appeal of this technique is that it is easy to see how it relates to the antisera themselves. As each serum is added to a cluster, the definition of that cluster is narrowed to include only those animals which

are positive for both the new serum and the cluster. This is fundamentally different to the way in which hierarchical clustering works.

A problem that is shared by both techniques is that they are poor at detecting sub-groups. It is common for the activity of one cluster to be entirely within that of another (Spooner et al, 1980; Spooner and Morgan 1981). It is not always obvious how this relates to the underlying antigens. If most animals are positive for both clusters then usually the clusters will be merged so that the new cluster is limited to animals positive for the sub-group. However, if the sub-group is rare among the members of the larger cluster but entirely confined to them, it would certainly be wrong to merge the clusters. This is because it is likely that there are two genuine antigens, the presence of the rarer being entirely dependent on the presence of the more common one.

There appears to be no simple solution to this problem. The computer algorithms that were used to perform the clustering in this study included a means of detecting such containment of one cluster within another (see Similarity Index programme below) but no attempt was made to use this information to influence the measure of similarity.

It was because of this difficulty that the computer was not used to perform the clustering entirely unaided. Instead, separate programmes were written which first

calculated the similarity index using (iii) above. The results of this analysis were scrutinised and used to form the input to the second programme which merged sera to create "phantom sera". Had these programmes been linked to perform a series of similarity index calculations followed by a merging of sera, the outcome would certainly have been different. It would have been necessary to have set thresholds of similarity at which to stop the clustering process. This would have forced the merging of some clusters which are probably true sub-groups, which would represent a major loss of information. For instance the w10, KMA003, KK4 complex would have been joined very early in the process to form a single cluster. Such an approach would also have completely failed to identify rare subgroups, such as the containment of KMA025 within the activity of the w6-defining sera, because of the low measure of similarity between these reagents which is entirely due to the relative rarity of animals positive for the sub-group.

Conclusion

Cluster analysis is an efficient way to extract information about antigens on PBL by examining the activity of alloantisera. Conventional clustering methods are unsuited to the analysis of typing reagents, but by formulating a new procedure to define clusters, useful information was extracted. A particular value of this method is its use during reagent development, perfect sera will be perfectly clustered by any method but the strategy described here, provides information which can lead to improvements. Even this approach does however have pitfalls and it is demanding of calculation time. In an attempt to minimise these problems, the analysis of the data, using the calculation of a similarity index and the clustering method described above, was performed by computer programmes whose output could be carefully scrutinised before proceeding.

Computing

Introduction

The data which are collected during the course of a series of BoLA-typing studies rapidly become difficult to handle. Typing lymphocytes from one animal against a panel of antisera generates a set of over 130 results without including such data as breed and sex. This data was required for a number of purposes the most demanding of which was the cluster analysis described above. It was therefore decided to use a computer for data storage and analysis.

The study had two major objectives; the investigation of the performance of the antisera and the antigens that they defined; and the study of the variations in BoLA antigens between different populations of cattle. The computer programmes were designed to assist these two studies. However a useful facility which the computer made available was the rapid storage and retrieval of data on individual animals.

The programmes were first written in FORTRAN IV and run on a PRIME 750 mini-computer. They were subsequently re-written for an IBM PC XT micro-computer. The programmes for the IBM were considerably more useful than those on the PRIME and so only these will be described. It is worth pointing out that only five years ago, programmes of such complexity could not have been satisfactorily run on any but large computers such as the

PRIME, and that the sudden appearance of inexpensive micro-computers with sufficient power for applications such as this one may be expected to revolutionise the process of data collection and analysis. These "micros" make it possible to move away from large mini or main-frame computers which require teams of specialists to maintain and programme them. It also means that processes controlled by computers in the laboratory become practicable; this is well illustrated in the field of the MHC by the appearance of a fully automated system for reading the lymphocytotoxicity test (Bruning et al, 1982; Naipal et al, 1984).

Hardware and commercial software

The micro-computer used was an IBM PC XT with 640 K bytes of random access memory (RAM), a fixed disk of 10 M bytes capacity and a floppy disk drive of 360 K bytes. The large disk capacity enabled manipulation of the very large data files which the study accumulated (typically of the order of one M byte). In addition, the fast access time of the hard disk in comparison to a floppy drive allowed the programmes to run efficiently. The large RAM capacity was utilised by means of commercial RAM drive simulation software (Superdrive, AST Research Inc.) and print buffer software (Superspool, AST Research Inc.).

An Epson FX-100 printer was connected to the computer, and the programmes which wrote their output to the printer (either directly or by first writing to a file

for subsequent spooling) provided the choice of a large number of print styles. This allowed a maximum of 233 characters per line (often required by the printout programme) when using condensed print, or fewer and larger characters when less columns were required. The print buffer programme allowed applications which generated large amounts of print-out, such as the similarity index programme, to run continually without waiting for the printer to finish.

Since the Pascal compiler could access only 128 K bytes of RAM, the large data arrays necessary for the clustering programmes could not be held in this space. One solution to this problem would have been to write assembly language routines to place the data in specific locations. This however would have been rather time consuming and tedious, so instead random access work files were written to the RAM drive. They were then written to and read from as if they were conventional arrays of data.

Programmes were written using a word processing package (Wordstar, Micro Pro International) and compiled by the Prospero Pascal compiler (Version 1.1; Prospero Software).

A listing of the source code of these programmes is contained in appendix two.

The_BoLA_analysis_Programmes

The programmes for the IBM were written in Pascal (Wirth, 1976). Pascal has a number of advantages over FORTRAN for such an application. It allows the definition of extremely complex data structures which makes it easy to manipulate data which is grouped in mixed data types. The information on each animal was stored as a variable with the following type declaration:

```
Rec_of_Results=RECORD
                 Name:Packed Array[1..40] of CHAR;
              Details:Packed Array[Set_of_fields] of
                      Packed Array[1..8] of CHAR;
               Serums:Array[1..240] of CHAR;
                 END;
```

Here the type "set_of_fields" was defined as:

```
TYPE Set_of_fields =
                  (batch, date, sex, breed,
                   location, subloctn,CBTS, Spare);.
```

The identity of the animal was held in the "name" field which allowed 40 characters, and thus could hold a great deal of information other than a simple identity number. The details of the test, the breed of the animal and the population from which it came, were held in the "details" field which was itself composed of eight fields each capable of holding up to eight characters. The reactions of the lymphocytes against each of the typing antisera was held in the "Serums" field which could hold up to 240 scores. This entire data structure occupied 344 bytes so that data from more than one thousand animals would onto a single floppy disk of 360 K bytes capacity.

Pascal also allows the definition of files with particular structures. In the case of the BoLA programmes, the files which held the typing information were defined as being of type "Rec_of_results". This allowed simple and rapid reading and writing of the complex data set defined above. Most of the programmes used a large number of sequential read and write operations. Since Pascal allows direct access to the file buffer, the programmes ran considerably faster than would have been possible under a language such as FORTRAN.

The superiority of Pascal over FORTRAN for this application is well illustrated by the Pascal code shown in appendix two (programme "convert FORTRAN to Pascal") which is designed to read data from a file formatted for reading by the FORTRAN programme. Because FORTRAN has no means of defining complex structures such as the "Rec_of_Results" used here, each data type must be handled separately and some 35 lines of complex code are required. Having defined the variable "Results" and the file "Pasfil" as of type "Rec_of_Results", the same end result is achieved for Pascal data by the lines:

```
WHILE NOT eof(Pasfil) and (X<=Copy) DO
BEGIN
  Read(Pasfil,Results);
  X:=X+1;
end;
```

Whether or not this generates faster machine code depends on the compiler, but it is certainly much less prone to error.

The programmes required a number of control files containing further information. The most important of these was called the "Batch" file. This held a sequence of serum names which allowed the programmes to assign a given reaction to a given serum. The batch file was a text-file which allowed changes in the serum names or sequence, to be made using a text editing programme. The two other control files, which were also text-files, are described later.

Eleven programmes were written, plus a simple "menu" programme to allow easy access to them.

The data-entry programme:

The data were entered using the "Entry" programme which collected data in a form that allowed direct reading of plates from the microscope onto the computer. It checked that the serum scores were within the set of valid scores. The NIH scoring scheme (Ray et al, 1977) was used with the modification that negatives were recorded as "-" and unreadable wells as ".", which made a printout of the data easier to read. The programme identified the plate and the sera which were being recorded and gave several opportunities for data checking and correction. To improve the speed and accuracy of data entry, several of the keys had their meanings modified by the programme. The zero key for instance entered a series of six negative scores; the F9 key a series of six not tested scores; and

the "+" key became an extra RETURN conveniently placed for the number pad.

The Update programme:

One possible disadvantage of using a Pascal non-text file to store data is that it cannot be corrected or modified by a conventional text-editor. Using such an editor would have been a hazardous operation anyway, as an alteration to the length of any of the fields would have caused the programme to fail and it would also have been extremely difficult to locate a given serum score or identity field.

Having decided to use a non-text data file, it became necessary to write a programme to maintain it. This programme, called "Update" also provided a review of the serum reactions and the identity of any of the animals in a file. It was used as much to provide rapid retrieval of information on individuals as to edit the data. Records were "deleted" from the data set by assigning the symbol for hexadecimal 23 to the first character of the name field by means of the update programme. Any such record was completely ignored by all of the programmes which performed analysis of the data.

If the data file to be manipulated by this programme was held in the RAM-drive, execution was extremely fast, but this was only possible with files less than 360 K bytes in length. In many cases, therefore, the programme read the records from the hard disk and was therefore

rather slow to search a long file. Since this programme was not designed as a database application, no attempt was made to overcome this drawback. There are a number of ways to dramatically speed up the search of a data file. However, they all require the setting up, either in RAM or on disk, of some form of index. Since most searches of a data file were "one off", it was not felt that this would be worthwhile in view of the significant time that would be required to prepare the index when the programme was first loaded.

The "utility" programmes:

Four short programmes were written to further manage the data. One of these converted data in the format used by the FORTRAN programmes to that used by the Pascal programmes while another reversed this process. The third simply copied a given number of Pascal records from one file and appended them to another, this allowed files to be split (by copying only some of the records) and joined as required. This was often necessary when transferring large files from the hard disk onto a series of floppy disks and vice versa.

The fourth of these programmes re-ordered the serum reactions in a data file, so that data collected at different times using different serum sequences could be merged or compared. As new sera were added to the standard panel, this programme was used to maintain a single, standardised data file. Records of animals which

had not been tested with the new sera were automatically given the "untested" score for their reactions with these sera.

The Selection programme:

In order to compare the BoLA types of different populations it was necessary to be able to create files containing details of all animals with given characteristics. This was achieved by the "selection" programme. It allowed records to be copied from one file to another depending on either the presence or absence of any characteristics in any of the data fields.

Thus, a file of Borans and a file of Sahiwals could easily be created. It was also useful to be able to form files containing data from animals with given serum reactions, and in conjunction with the "printout" programme, this provided a simple way of examining the correlation between different antisera.

Up to 50 different character strings in any field could be selected for in one pass through the data. Selection could either be on the basis of an exact match with the string being searched for, or it could be by treating it as a sub-string so that records would be selected if the string in the relevant field contained the appropriate sub-string. This was a useful means of collecting data on groups of animals with common components to their identifying fields. It also allowed

the space available for data storage to be used more efficiently, since several independent pieces of information could be stored in a single field provided that a convention was used to allow them to be distinguished. The simplest example of this was the use of the character string "LCL" in the name field to denote a cell line.

The Printout programme:

To display the information on individuals the "printout" programme was used. It printed the condensed identity details of each animal, together with the reactions of its lymphocytes with up to 187 antisera (this was limited by the maximum number of characters the printer could write on one line). An example of the output of this programme is shown in Figure 4.1

The order in which the serum reactions were displayed was determined by the second "control" file, the sequence file. As well as determining the sequence of the sera whose reactions were displayed in the output, this file also governed the positioning of both the headings for groups of antisera, and of the symbols used to divide the output into convenient groups of sera. This file was extremely simple to re-arrange, so sera could be "moved about" on the print-out at will. The programme provided a powerful means of judging the relationships between the reactions of antisera, which could then be tested by calculating a similarity index. It also gave a useful

Print out of file B:BIT.DTA
Serum batch is 6CLS3.BCH
Using serum sequence CLSTR.SEQ

prepared 5/FEB/1984

NAME	DATE	BREED	LOCTN	SEX	W1	W3	W2	W4	W5	W6	KM18	W7	W8	W10	W11	W12
					EEEE	EE	EEEEE	E	EEE	EE	EEEEEK	MKKM	EEEEK	EEEK	EMEEE	KKM	EEEE	EE
					DDDD	FF	DDDDD	D	FFD	DF	DDDDDM	AMMA	DFDDM	DDDM	DADDD	MKA	DDDB	FF
					AAAA	AB	AAAAA	A	ABA	AA	AAAAAA	0AA	AAAAA	AAAA	A0AAA	AA0	AAAA	AB
					0001	00	00001	0	001	00	000000	500P	00000	0000	17000	004	0011	00
					0081	33	06661	8	881	09	016793	.113	11660	1682	0.679	00.	7701	44
					1832	99	22455	5	227	70	906578	108	17787	3302	51916	341	3620	77
B166	02-09-83	BORAN	ILRAD	F	----	--	-----	8	888	--	------	.--.	-2---	----	8.-88	8..	----	--
B171	02-09-83	BORAN	ILRAD	F	----	--	----8	8	---	--	---8-	-.-8.	88888	----	-.---	-..	----	--
B626	02-09-83	BORAN	ILRAD	M	----	--	-----	-	---	--	------	.--.	-----	8---	-.---	-..	----	--
B631	02-09-83	BORAN	ILRAD	F	----	--	-----	-	---	--	----8-	-.-8.	-----	----	8.---	-..	----	--
C237	08-09-83	BORAN	ILRAD	M	----	--	-----	-	---	--	------	.--.	-----	88--	-.---	-..	----	--
C210	08-09-83	BORAN	ILRAD	M	----	--	-----	4	---	--	888888	.--.	-----	----	8.---	-..	----	.-
C230	08-09-83	BORAN	ILRAD	M	----	--	-----	-	---	-8	------	.--.	-----	----	8.--6	-..	----	--
C156	08-09-83	BORAN	ILRAD	F	----	-.	---4-	-	---	--	888888	.--.	-----	----	8.--6	-..	----	--
C165	08-09-83	BORAN	ILRAD	M	----	--	-----	6	---	--	888888	.--.	88888	----	8.--8	-..	----	--
C167	08-09-83	BORAN	ILRAD	F	----	--	-----	8	886	--	------	.--.	-8-8-	----	8.688	-..	----	--
C91	08-09-83	BORAN	ILRAD	M	----	--	-----	-	---	--	------	.--.	-----	----	6.---	-..	----	--
C241	08-09-83	BORAN	ILRAD	M	----	--	--8--	8	---	--	------	.--.	-----	-6--	-.---	-..	----	--
C235	08-09-83	BORAN	ILRAD	M	----	--	--8--	8	---	--	------	.--.	-----	88--	-.---	-..	--.-	--
C166	08-09-83	BORAN	ILRAD	F	----	--	--8--	-	-.-	--	888888	.--.	---8-	88-8	8.---	-..	----	--
C238	08-09-83	BORAN	ILRAD	M	----	--	-----	-	---	--	------	.--.	-----	8---	-.---	-..	----	--
C242	08-09-83	BORAN	ILRAD	M	----	--	-----	-	---	--	------	.--.	-----	----	-.---	-..	----	--
C253	08-09-83	BORAN	ILRAD	M	----	--	-----	8	---	--	------	.--.	-----	----	8.-88	-..	----	--
C296	08-09-83	BORAN	ILRAD	M	----	--	-----	8	888	--	------	.--.	-----	----	8.---	-..	----	--
C275	08-09-83	BORAN	ILRAD	M	----	--	-----	-	---	--	------	.--.	-----	----	-.---	-..	----	--
C248	08-09-83	BORAN	ILRAD	M	----	--	-----	-	---	--	888888	.--.	-----	----	8.---	-..	----	--
C234	08-09-83	BORAN	ILRAD	M	----	--	-----	8	---	--	8-8888	.--.	-8---	----	8.888	6..	----	--
C244	08-09-83	BORAN	ILRAD	M	----	--	-----	-	---	--	---4--	.--.	-----	88-8	-.---	-..	----	--
C236	08-09-83	BORAN	ILRAD	M	8888	--	-----	-	---	--	------	.--.	-----	8---	-.---	-..	----	--
C209	08-09-83	BORAN	ILRAD	M	----	--	-----	-	---	--	868888	.--.	-----	6---	8.---	-..	----	--
C229	08-09-83	BORAN	ILRAD	M	----	--	-----	8	---	--	------	.--.	88888	----	-.---	-..	----	--
C245	08-09-83	BORAN	ILRAD	M	----	--	-----	-	---	--	------	.--.	-----	4---	-.---	-..	----	--
C292	08-09-83	BORAN	ILRAD	M	----	--	-----	-	---	--	888886	.--.	-----	8---	8.---	-..	----	--
C295	08-09-83	BORAN	ILRAD	M	----	--	-8888	8	---	--	------	.--.	88888	----	-.---	-..	----	--
C232	08-09-83	BORAN	ILRAD	M	----	--	-----	8	---	-6	------	.--.	-----	----	8.-88	-..	----	--
C331	14-09-83	BORAN	ILRAD	M	----	--	-----	8	---	--	888888	.--.	-88--	----	8.888	8..	----	--
C330	14-09-83	BORAN	ILRAD	M	----	--	-----	-	---	--	------	.8-.	-----	----	-.---	-..	----	--
C329	14-09-83	BORAN	ILRAD	M	----	--	-----	-	---	--	888888	.--.	-----	88-8	8.---	-..	----	--
C326	14-09-83	BORAN	ILRAD	M	----	--	-----	-	---	--	------	.--.	-----	----	-.---	-..	----	--
C231	14-09-83	BORAN	ILRAD	M	----	--	-----	-	---	--	888888	.--.	-----	4---	8.---	-..	----	--
C233	14-09-83	BORAN	ILRAD	M	----	--	-----	8	886	--	------	.--.	-----	4---	8.---	-..	----	--
C294	14-09-83	BORAN	ILRAD	M	----	--	-----	-	---	--	------	.--.	-----	86--	-.---	-..	----	--
C252	14-09-83	BORAN	ILRAD	M	----	--	-----	-	---	--	------	.--.	-----	----	8.---	-..	----	--
C293	14-09-83	BORAN	ILRAD	M	----	--	-----	-	---	--	------	.--.	-----	----	-.---	-..	----	--
B166	14-09-83	BORAN	ILRAD	F	----	--	-----	8	888	--	------	.--.	-----	----	8.-68	-..	----	--
B732	16-09-83	BORAN	ILRAD	F	8---	--	-----	8	---	--	888888	.--.	-8---	----	8.888	4..	----	--

Fig 4.1 Sample from PRINTOUT programme

summary of the BoLA types of individuals or groups of animals.

The Similarity Index programme:

A more precise means of comparing antisera was possible by calculating the similarity index (described above) between them. The similarity index of serum s1 and serum s2 was defined as % SIMTY=100(2a/(2a+b+c)) where a, b, and c are values in the 2x2 table above.

The programme treated all serum scores as positive or negative (or untested). However since the actual score recorded for the serum was available in the data file it was possible to choose to treat the partial score "4" as either positive or negative. In practice, this made little difference to the clustering as most antisera generated few such scores.

The programme calculated these values for a given serum as s1 with each of the sera against which the animals in a file had been tested as s2. The minimum value of the similarity index to be printed out was chosen by the user, as was a minimum value above which the output was highlighted by a bold print face. The similarity index is rather poor at identifying antisera whose reactions are entirely within those of another (and correlation coefficient is considerably worse). If there are relatively few positive reactions against the less reactive antiserum (as happens in rare sub-groups) then

they will show low correlations. To overcome this, a series of simple tests was performed on the data by the programme. It established four levels of such containment:

level 1 - no evidence of containment

level 2 - either b or c are less than 10% of a

level 3 - level 2 and where a was greater than 10

level 4 - level 3 and where b or c was zero.

This programme required four random-access work files to hold the totals for each of the elements of the 2x2 table for every combination of antisera. These files were declared as "FILE OF WORKTYPE"; where the type "WORKTYPE" was declared as;

"WORKTYPE=Array[1..240] of two_byte"

and; "two byte" as the range 1..65534. This limited the number of animals that could be clustered to 65534. However, it seemed unlikely that this quantity would be approached, and it allowed the files to fit into the available RAM. Each file was initialised by the programme up to a maximum of 240 records, so that a complete clustering of 240 sera could be performed.

The files were treated as large arrays, a particular record being found by the "SEEK" statement and its contents examined or altered by a statement such as "WORK_FILE^[serum]:=WORK_FILE^[serum]+1". These files were always located on the RAM drive, since hundreds of thousands of such statements were executed during a

programme run. A conventional drive would have been impossibly slow.

The gene-frequency comparison programme:

Another programme calculated antigen frequency, gene frequency and standard deviation for every antiserum in a population. Gene frequency was defined as:

$gf = 1 - \sqrt{(1-\text{antigen frequency})}$ (Mattiuz et al, 1970). This assumes Hardy-Weinberg equilibrium in the population. Standard deviation was conservatively estimated by $sd = \sqrt{gf/2N}$ where N is the number of individuals in the population (Pickbourne et al, 1978).

The programme calculated these values for two populations and then calculated the chi-square value for their antigen frequencies, the null hypothesis being that there was no significant difference between them. It also summed the chi-squares of each antiserum to give an estimate of the difference in antigen frequencies between the populations.

All sera were treated as if they were detecting single antigens. Obviously this is not true in many cases, but it provided flexibility by allowing clusters to be treated in the same way as single sera.

The Cluster programme:

Finally, a programme was written which allocated extra reactions to the records in a file according to the reactions of clusters of antisera. That is, it added scores corresponding to the presence or absence of antigens detected by "phantom sera". The phantom sera being defined in terms of real sera by the user, who made these decisions on the basis of the output from the similarity index programme.

The programme could assign up to 40 clusters, each holding up to 40 antisera. It was used to compare the performance of the antisera by allowing a similarity index to be calculated between these clusters and the defining sera. The clusters were defined by a control file which was easily changed to investigate the effects of varying the way in which clusters were allocated. This programme also allowed the gene-frequency and antigen frequency of clusters of antisera to be calculated and compared between populations.

Conclusions

The programmes described were used to analyse the data collected during the course of the study, and proved to be of immense value both in the characterisation of typing reagents and in the investigation of the BoLA antigens themselves. However, perhaps even more important than their use in the final analysis of the data was their value in the continuous process of serum preparation and refinement. They allowed the rapid and accurate identification of panels of animals to test absorptions, to make grafts and to test hypotheses. They also provided a data-base for work in such areas as T-cell killing, where details of the BoLA phenotype of experimental animals and cell-lines were needed.

It is certainly true to say that a major contribution to this study (and to several others) was made by the programmes described, they are constantly being refined and their value is likely to increase. There are no reports in the literature of comparable tools, the analyses performed at the HLA comparison tests for example are performed on main-frame computers (Albert *et al*, 1984) and in some ways are more powerful than those described here, but they are much less flexible and are not available to the worker as he develops his typing reagents. If simple programmes are available for constant use throughout the working time of a laboratory then the quality of the typing reagents will be raised and the complexity of the final analysis will be reduced.

Chapter 5

SUB-GROUPS OF w6, w8 AND w10

Introduction

A major feature of the results of the BoLA-typing was the appearance of previously unknown sub-groups of existing specificities. The three specificities which showed this behaviour were w6, w8 and w10. Such splits are an extremely common feature of HLA serology, where, for example, 13 of the 23 HLA-A locus products are splits of others (Albert *et al*, 1984), but with the exception of the w6 sub-groups, they have not been prominent in BoLA-typing.

It seems clear from the HLA data that a split is usually due to common determinants shared by several alleles, so that "splitting" a specificity amounts to distinguishing different, but serologically related, products (Lopez de Castro *et al*, 1984). An alternative explanation for a split however, is the presence of closely linked loci and this was proposed by Spooner and Morgan (1981) as a possible explanation for the w6 subgroups. Products of linked loci showing a sub-group relationship might be expected to be a more common phenomenon in BoLA-typing than in HLA-typing, because of the widespread use of artificial insemination in cattle to propagate a limited number of haplotypes throughout a herd. For such a sub-group relationship to persist in herds in East Africa, West Africa and Europe would however

require an extremely high linkage disequilibrium in a two locus model.

The splits of w6 which were seen in this study were not unexpected, for although w6 is a common and well defined specificity, the first international workshop provided evidence that three antisera were detecting sub-groups of w6.

An extensive study of w6 was made by Spooner and Morgan (1981). They made a series of cross-absorptions of the four Edinburgh w6 defining sera, and showed that three of them were not operationally monospecific. EDA009 could be absorbed with lymphocytes from any one of a group of w6 animals, and be left with reactivity for another group. EDA066 behaved in a very similar way, while EDA075 appeared to contain different specificities. EDA010, on the other hand, contained only activity for the broad w6 specificity ("w6-broad").

At the second workshop w6-broad was defined by 14 antisera which reacted with cells from 39 animals. In addition, two sub-groups were defined, w6.1 and w6.2. w6.1 was defined by five sera from Edinburgh and France, while w6.2 was defined by three sera from Edinburgh and Texas.

The European workshop (INRA, 1982) confirmed w6, w6.1 and w6.2. Subgroups w6.3 and w6.4, as described by Spooner and Morgan (1981), appeared to be present but could not be confirmed.

Another cluster which showed evidence of sub-groups in this study was w8. Evidence that w8 does have sub-groups has been put forward at the international workshops. At the second workshop it was suggested that a serum from Texas which appeared not to detect all of the w8 animals, was detecting a w8 sub-group, but the evidence for this was limited. Spooner et al (1980) also presented some evidence for a w8 sub-group. The European workshop of 1982 also suggested that w8 may contain a sub-group. This suggestion was based on the fact that there appeared to be some w8 cells which did not react with the serum EDA080. However, since all but two of the 15 defining sera showed non-w8 reactions, it is hard to draw such a conclusion from the absence of activity for two cells.

w10 was defined at the first workshop by seven sera from five laboratories. There was good evidence of at least one sub-group. At the second workshop it was defined by five sera from three laboratories. Again, there was evidence of a sub-group. The European workshop confirmed w10 with four animals and four antisera. There was no evidence of a sub-group here, no American sera were tested and only sera from American laboratories have shown this activity in the past.

Therefore, although Spooner et al (1980) suggested that many of the BoLA specificities, in addition to w6, were supertypic, there was hitherto little evidence to support this. The appearance of new subgroups in two

important specificities is thus a significant development.

Methods

Cluster analysis.

For the purpose of cluster analysis, the data were first divided into three sets. These were the results of typing 606 animals of "Zebu" type, the results of typing 662 Borans and the results of typing 351 *Bos taurus* animals in Edinburgh. These groups are fully described in table 2.1. Not all of the available typings were used in the cluster analysis, since some were from populations in which few animals were typed and the numbers were felt to be too low to be useful for this purpose.

All of these results were stored on the computer using the entry programme. The populations were separated by the selection programme and similarity index calculations made by the similarity index programme.

The preliminary analysis did not make any assumptions about the way in which the sera would cluster. The similarity index between each serum and the entire serum panel was calculated. The threshold for recording the result of each calculation was set at a similarity of 10%, this allowed many small associations to be recorded but it ensured that no important results were missed. It was anticipated that there would be differences in the performance of the sera between the populations, so it was felt that it would sometimes be of value to record non-

significant results because they may reach significance in other populations.

On the basis of this analysis, the European sera were formed into clusters and the cluster programme was used to add the clusters to the results of each animal. The similarity index programme was then re-run on this data. In this way it was possible to form an accurate picture of the performance and inter-relationships of the sera and the workshop clusters.

Finally, serographs were prepared using the printout programme, with the serum sequences determined by the process described above. This gave a clear picture of the performance of the sera that had been clustered.

Significance

It is not useful to select a given value of similarity index as the threshold between significant or non-significant. This is because the value reached is not influenced by the number of animals tested, and so offers no guidance on the reliability of the result. It is also relatively meaningless under a number of circumstances, notably in the case of rare subgroups. More useful in many cases are the raw values from the 2x2 table. The similarity index was therefore only used as a guide to associations which might be significant. During the analysis by the computer a cut-off-point for printing details of sera was set at 10% similarity, this together

with the algorithms which searched for evidence of sub-groups regardless of the similarity index ensured, that no potentially important data was missed.

Something of the difficulty in extracting information from a similarity index value is illustrated by the histograms of Figure 5.1 which shows data from the Boran population. The first shows the number of sera in each class of similarity with KMA038, a typical good monospecific serum detecting a well defined cluster - w6. Here, a cut-off of 90% would clearly allow the definition of a very close cluster because there are few non-w6 reactions of KMA038 and few sera which react with w6 and other antigens.

In contrast to this are the figures for the similarity indices of a poor and probably multispecific serum, EDA105. The similarity of this serum is not above 70% with any other serum, and there is a spread of values from zero to this figure. However, this serum is useful in the definition of w10, and a cut-off above 10% would have meant the loss of useful information.

Even more disturbing are the figures for EDA085. They were almost identical to those of EDA105 and yet EDA085 is monospecific but detects a supertypic specificity (common to three alleles; w4, w7 and w10). This serum had no associations above 60%; into the 50-60% class fell the sera detecting w7; those detecting w4 were

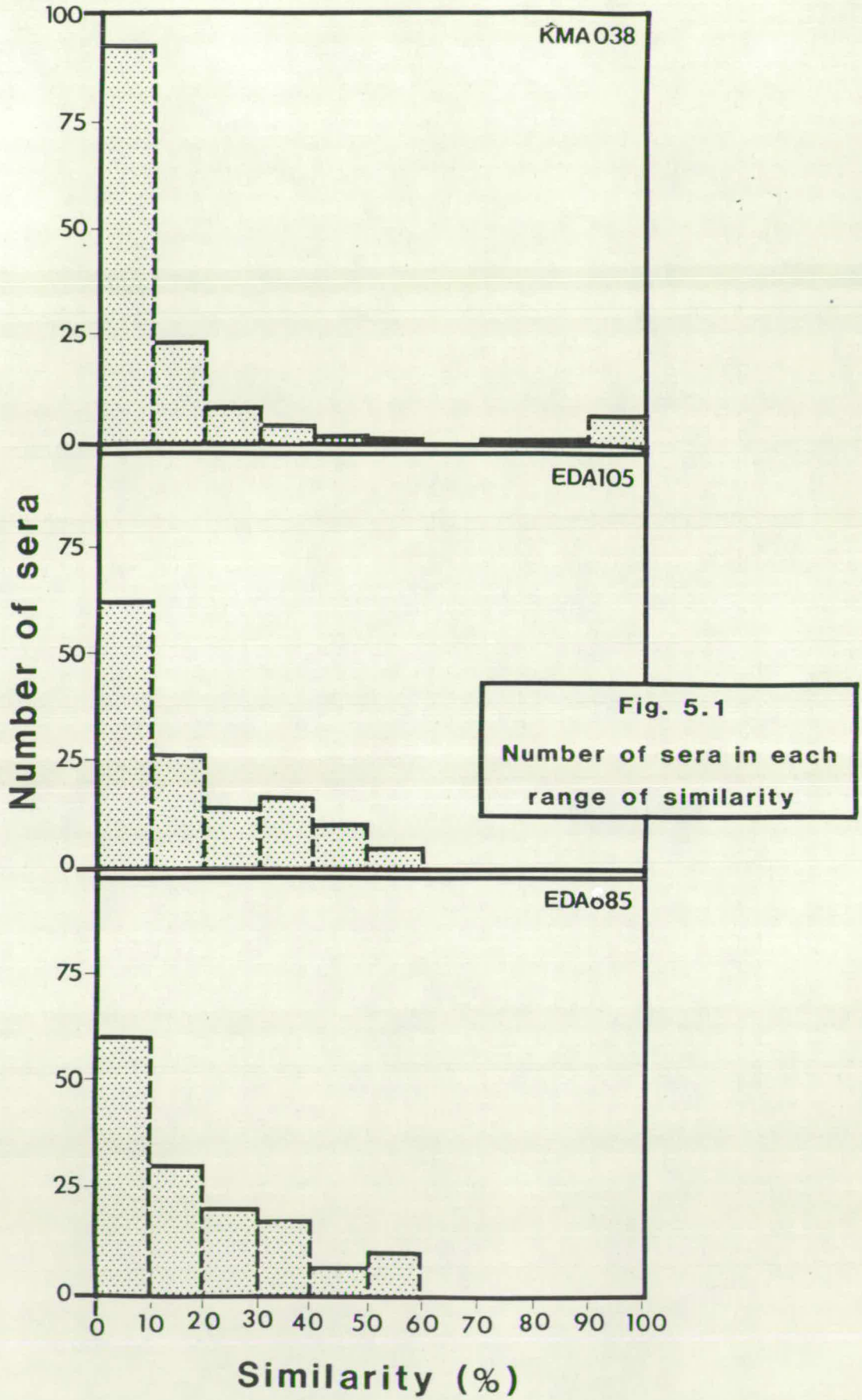


Fig. 5.1

Number of sera in each range of similarity

in the 40-50% class, and those detecting w10 were in the 30-40% class. These differences result entirely from the different frequencies of the three alleles w4, w7 and w10, and another allele confined to EDA085 positive animals could show any value from almost zero if it were rare.

The similarity index was therefore no more than a useful guide. However, Figure 5.1 suggest that most pairs of sera with similarity indices less than 30%, and which do not show evidence of a sub-group relationship, are not sufficiently related to be useful for antigen definition. This could not, however, be considered to be a hard and fast rule, hence the decision to set the initial cut-off point for the analysis at a safe 10%.

Nomenclature

Throughout this and the following chapters, a number of conventions have been adopted to avoid confusion.

An antiserum always has six characters in its name, for example KMA017. When a cluster is formed from the coincidence of two antisera, its name never has six characters but is derived from the name of the antiserum with the activity which most closely corresponds to that of the cluster. The term "phantom serum" and "cluster" are used interchangeably and have the same meaning, a "phantom serum" being defined by a "cluster" of alloantisera. For example, the cluster whose definition includes the antiserum KMA017 is called KM17. In

accordance with convention, a cluster name refers not to the reagents which detect a specificity, but to the specificity itself. Thus a cell expressing KM17 reacts with the sera KMA017 and KMA029.

The tables showing similarity indices are all of the same form. For economy of space the key is given only for the first such table (w6, table 5.1).

Results

The sub-groups of w6

The initial similarity index calculations on the w6-defining sera showed a group of five extremely highly correlated sera in all populations. The correlation between all combinations of these sera was of the order of 90%.

The analysis was repeated using a cluster defined by the overlap of the four Edinburgh sera of this group: EDA009, EDA010, EDA066 and EDA075. Table 5.1 shows the results of the analysis following this assignment of w6. It shows the close correlation between the w6 defining sera in all populations. This cluster was unique in that there was no loss of specificity in its defining reagents in the African cattle.

The serum EDA097 clustered well with the w6 sera in the European animals, but in both the Zebu and Boran groups it had a large number of reactions with non-w6 animals. Most of these animals had the KM18 specificity (described below), but there remained some reactions of EDA097 with animals which were neither w6 nor KM18. Serograph Figure 5.2 summarises this situation.

The Kenyan serum KMA025 appeared to detect a previously unknown sub-group of w6. In the Zebu group, the only six reactions of KMA025 were confined to w6 positive animals. These six animals, however, came from

one herd and there is a possibility that they were related.

The sera which have been assigned by international workshops to the w6 subgroups are as follows; w6.1: EDA012, EDA092, EDA093; w6.2: EDA086, EDA081. Spooner and Morgan (1981) further define w6.3 detected by EDA087, and w6.4 to which EDA100 was provisionally assigned by the European workshop.

These assignments cannot be upheld by the results of the Bos indicus populations. In the European population, the similarity indices between the w6.1 defining sera were higher than with any other sera, and their activity was largely confined to w6 positive animals. The same applied to the w6.2 reagents with the addition that EDA113 was closely associated with this cluster.

Following these initial calculations a further clustering was performed using the above definition of w6.1 and defining w6.2 as EDA081, EDA086 and EDA113. The results of this analysis are contained in tables 5.2 and 5.3. w6.1 formed a cluster in the European animals, with EDA093 showing more activity than the other sera. The frequency of the specificity detected by this cluster was too low for useful comment in the Borans, and in the Zebus it was seen in nine animals. However, these sera clustered less closely in the African animals than in the European breeds. The cluster KM18, which is detected by the sera KMA010 and KMA018, clustered with w6.1 in the

Table 5.1: w6-broad

	1	2	3	4	5	6 **
	SERA		NUMBER		+VE	SIMTY
population	S1	S2	S1	S2	S1&S2	(%)
Zebu	W6	EDA009	0	12	79	92.9
	W6	EDA010	0	5	79	96.9
	W6	EDA012	29	13	49	70.0
	W6	EDA066	0	7	79	95.8
	W6	EDA075	0	7	79	95.8
	W6	EDA097	2	57	77	72.3
	W6	KMA025	73	0	6	14.1
	W6	KMA038	1	5	78	96.3
Boran	W6	EDA009	0	5	155	98.4
	W6	EDA010	0	1	155	99.7
	W6	EDA012	44	9	111	80.7
	W6	EDA066	0	11	155	96.6
	W6	EDA075	0	9	155	97.2
	W6	EDA081	23	8	132	89.5
	W6	EDA091	53	87	102	59.3
	W6	EDA097	1	42	153	87.7
	W6	EDA098	112	30	43	37.7
	W6	EDA105	38	255	117	44.4
	W6	KMA038	4	4	146	97.3
European	W6	EDA009	0	6	62	95.4
	W6	EDA010	0	4	62	96.9
	W6	EDA012	38	1	24	55.2
	W6	EDA066	0	5	62	96.1
	W6	EDA075	0	3	62	97.6
	W6	EDA081	30	4	32	65.3
	W6	EDA086	33	3	29	61.7
	W6	EDA092	41	4	21	48.3
	W6	EDA093	37	28	24	42.5
	W6	EDA097	1	7	61	93.8
	W6	EDA113	33	10	29	57.4
	W6	KMA038	8	12	53	84.1

**

1 The name of the cluster or "phantom serum" - S1.
2 The serum with which it is compared - S2.
3 The number of animals positive for the cluster but not the serum - value "b" in the 2x2 table.
4 The number of animals positive for the serum but not the cluster - value "c" in the 2x2 table.
5 The number of animals positive for both - value "a" in the 2x2 table.
6 The similarity index between these two "sera".

Table 5.2: w6.1

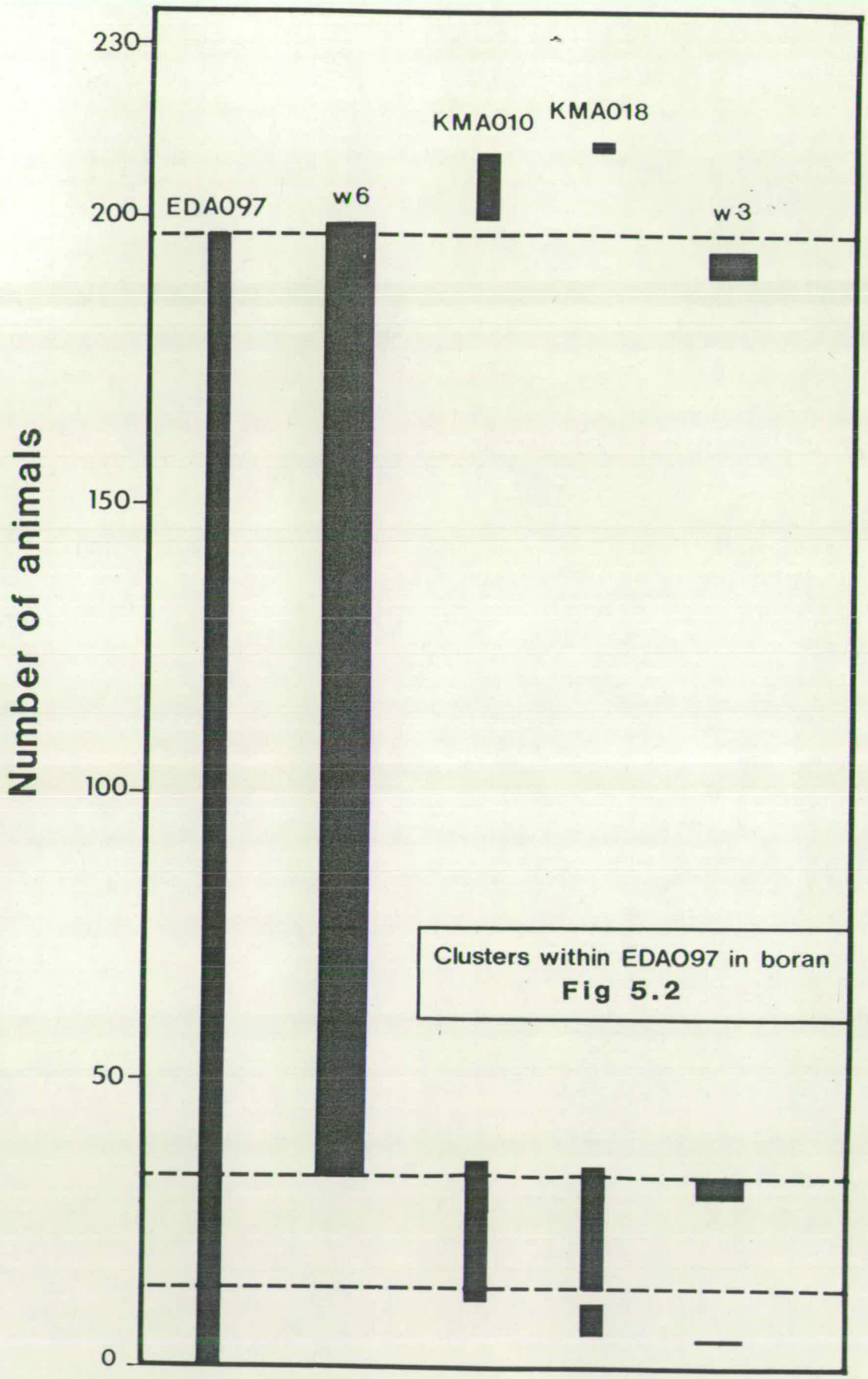
population	SERA S1	SERA S2	NUMBER +VE S1	NUMBER +VE S2	NUMBER +VE S1&S2	SIMTY (%)
Zebu	W6.1	EDA012	0	53	9	25.4
	W6.1	EDA092	0	20	9	47.4
	W6.1	EDA093	0	38	9	32.1
	W6.1	KM18	3	17	6	37.5
Boran	W6.1	OCCURS IN ONLY ONE ANIMAL				
European	W6.1	EDA009	0	54	15	35.7
	W6.1	EDA010	0	52	15	36.6
	W6.1	EDA012	0	10	15	75.0
	W6.1	EDA066	0	52	15	36.6
	W6.1	EDA075	0	50	15	37.5
	W6.1	EDA092	0	10	15	75.0
	W6.1	EDA093	0	37	15	44.8
	W6.1	EDA097	0	54	15	35.7
	W6.1	KMA010	3	7	12	70.6
	W6.1	KMA038	1	52	14	34.6
	W6.1	W6	0	47	15	39.0

Table 5.3: w6.2

population	SERA S1	SERA S2	NUMBER +VE S1	NUMBER +VE S2	NUMBER +VE S1&S2	SIMTY (%)
Zebu	W6.2 OCCURS IN ONLY ONE ANIMAL					
Boran	W6.2 OCCURS IN ONLY TWO ANIMALS					
European	W6.2	EDA009	1	38	30	60.6
	W6.2	EDA010	1	36	30	61.9
	W6.2	EDA066	1	37	30	61.2
	W6.2	EDA075	0	34	31	64.6
	W6.2	EFA081	0	5	31	92.5
	W6.2	EDA086	0	1	31	98.4
	W6.2	EDA097	1	38	30	60.6
	W6.2	EDA113	0	7	31	89.9
	W6.2	KMA038	4	39	26	54.7
	W6.2	W6	2	33	29	62.4

Table 5.4: The w6 subgroups

	Numbers of animals			
	(Total w6)		w6 +ve	w6 -ve
Zebu	(79)	w6.1	7	2
		w6.2	1	0
Boran	(155)	w6.1	0	1
		w6.2	1	1
European	(62)	w6.1	15	0
		w6.2	29	2


230
200
150
100
50
0
Number of animals
EDA097
w6
KMA010
KMA018
w3
Clusters within EDA097 in boran
Fig 5.2

Apparent w7/w10 cross-reactivity

Another cluster which showed an interesting behaviour was w7. In the European animals there was no evidence of an association between w7 and w10. Although in both European and African cattle w7 and w10 occurred only in animals also positive for the serum EDA085. However, in the African cattle, particularly the Borans, two of the w7 defining sera (EDA017 and EDA067) reacted without the other two in a large number of animals, the vast majority of which were also w10 (see Figure 5.3).

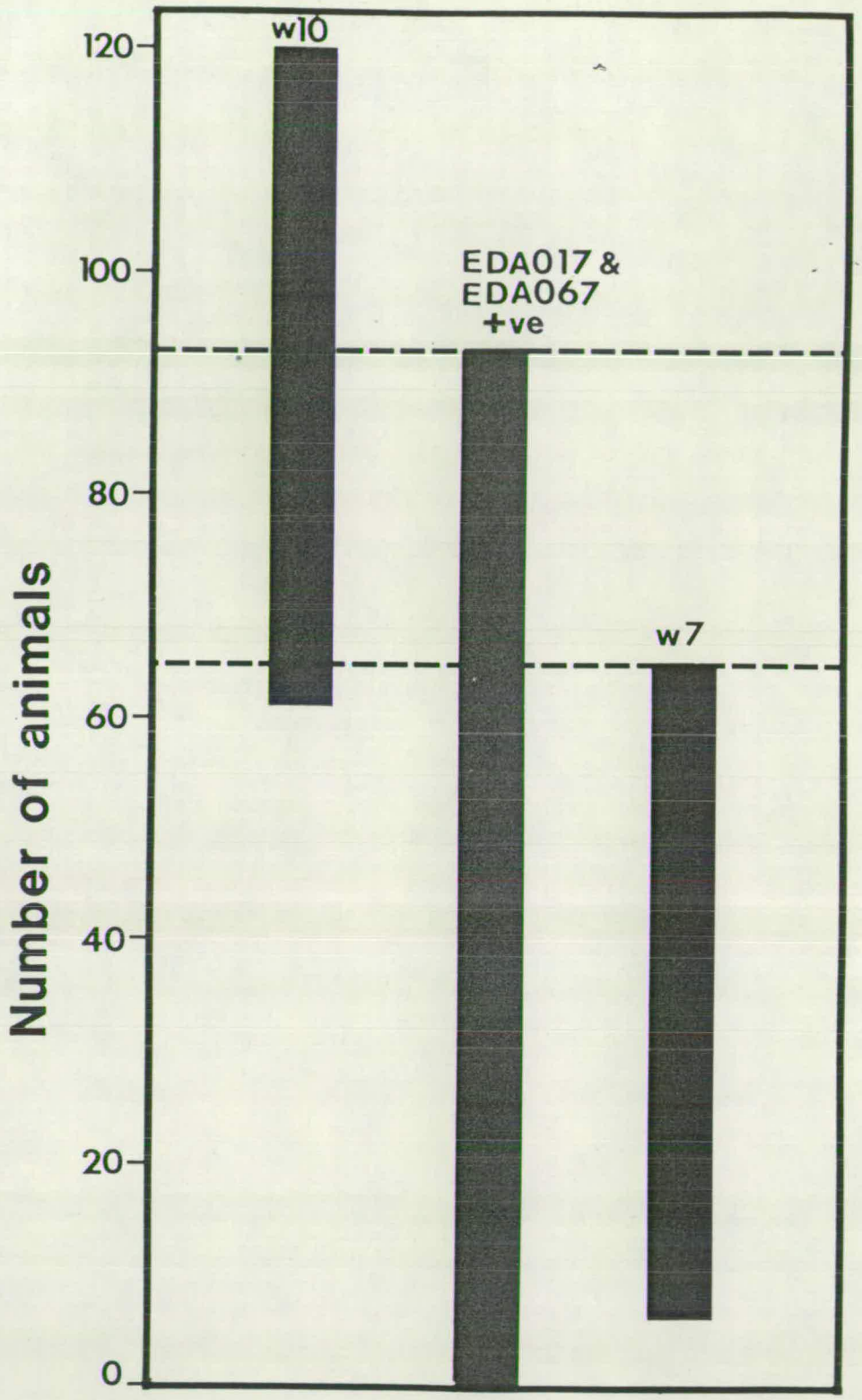
The cluster 7&10 was created, which comprised EDA017 and EDA067 together with the w10-defining sera, in order to study this phenomenon. Table 5.5 shows the similarity indices of this cluster, and its relationship with w7 and w10 is summarised in serograph Figure 5.3. This cluster occurred in only one animal in the European population - a w7/w10 animal - but in many animals in both of the African groups.

Table 5.5: 7&10

population	SERA S1	SERA S2	NUMBER +VE S1	NUMBER +VE S2	NUMBER +VE S1&S2	SIMTY (%)
Zebu	7&10	EDA017	0	92	8	14.8
	7&10	EDA067	0	112	8	12.5
	7&10	EDA069	0	28	8	36.4
	7&10	EDA071	0	52	8	23.5
	7&10	EDA085	0	279	8	5.4
	7&10	EDA096	0	242	8	6.2
	7&10	EDA105	0	243	8	6.2
	7&10	KMA003	3	2	5	66.7
	7&10	W10 *	0	15	8	51.6
Boran	7&10	EDA017	0	80	32	44.4
	7&10	EDA067	0	104	32	38.1
	7&10	EDA069	0	59	32	52.0
	7&10	EDA071	0	58	32	52.5
	7&10	EDA072	0	295	32	17.8
	7&10	EDA085	0	247	31	20.1
	7&10	EDA096	0	202	32	24.1
	7&10	EDA105	0	340	32	15.8
	7&10	KMA003	3	17	29	74.4
	7&10	KKA004	2	7	12	72.7
	7&10	MA04.1	3	13	15	65.2
	7&10	MA07.1	0	141	18	20.3
	7&10	W10	0	27	32	70.3
	7&10	M4 !	3	7	11	68.7
European	7&10	OCCURS IN ONLY ONE ANIMAL				

* w10 defined by EDA069, EDA071, EDA096.

! M4 defined by MA 4.1 and KKA004.



w7 and w10 in Boran

Fig 5.3

Evidence for sub-groups of w8

The initial analysis of the performance of the w8 defining sera showed a dramatic difference between the two Bos indicus populations and the Bos taurus animals. Two of the Edinburgh sera which have been assigned to w8 at the second international workshop, EDA013 and EDA063, had a similarity index of about 70% in the Bos indicus animals and 87% in the Bos taurus animals. However, EDA080 which has also been assigned to w8, showed no correlation with these two sera in the Bos indicus groups. In the Bos taurus animals this serum was negative for some cells which reacted with EDA013 and EDA063, but it still had a correlation of around 75% with them.

KMA022, a serum made in Kenya, appeared to detect a sub-group of w8 which occurred both in Bos indicus and Bos taurus animals. This serum was made in a w6/w10 Friesian, innoculated with Theileria parva infected lymphoblasts from a Friesian which was w6; EDA013, EDA063 positive; and EDA080 negative.

The cluster w8 was therefore defined as EDA013 and EDA063. Another cluster, w8+, was defined as EDA013, EDA063 and EDA080 in order to examine the relationship of EDA080 with the other two sera. A potential sub-group of w8 called KM22 was defined as EDA013, EDA063 and KMA022 in order to find any relationship between this sub-group and the other sera which appeared to be associated with w8. The similarity index analysis was then re-run with these

clusters, and the results of these analyses are summarised in tables 5.6, 5.7 and 5.8.

The correlation between the w8 defining sera and w8 was about 80% in the Zebus. In the Borans, EDA063 clustered closely (96.1%) with w8, that is, there were very few EDA063 positive animals which were not also EDA013 positive. In the Zebus, there was no correlation between EDA080 and w8, while in the Borans the relationship was weak, the majority of w8 animals being EDA080 negative.

The relationship between EDA080 and this cluster was very different in the European cattle, although EDA013 and EDA063 also correlated very highly (88% and 98% respectively) with w8. Most (20/36) w8 animals were EDA080 positive, which is in contrast to both Bos indicus groups where most are w8 positive and EDA080 negative. This is summarised in Figure 5.4.

There was a comparable proportion of EDA080 positive/w8 negative animals in the two groups but a large excess of w8 positive/EDA080 negative animals in the Bos indicus animals. This suggests a fundamental difference between the w8 in each group. The picture was confirmed by the behaviour of the cluster w8+ (table 5.7).

The cluster KM22 occurred in both w8 and w8+ animals in all three populations. Approximately half of the Borans with w8 were also KM22 positive, and approximately one third of the European w8 animals were KM22 positive.

Table 5.6: w8

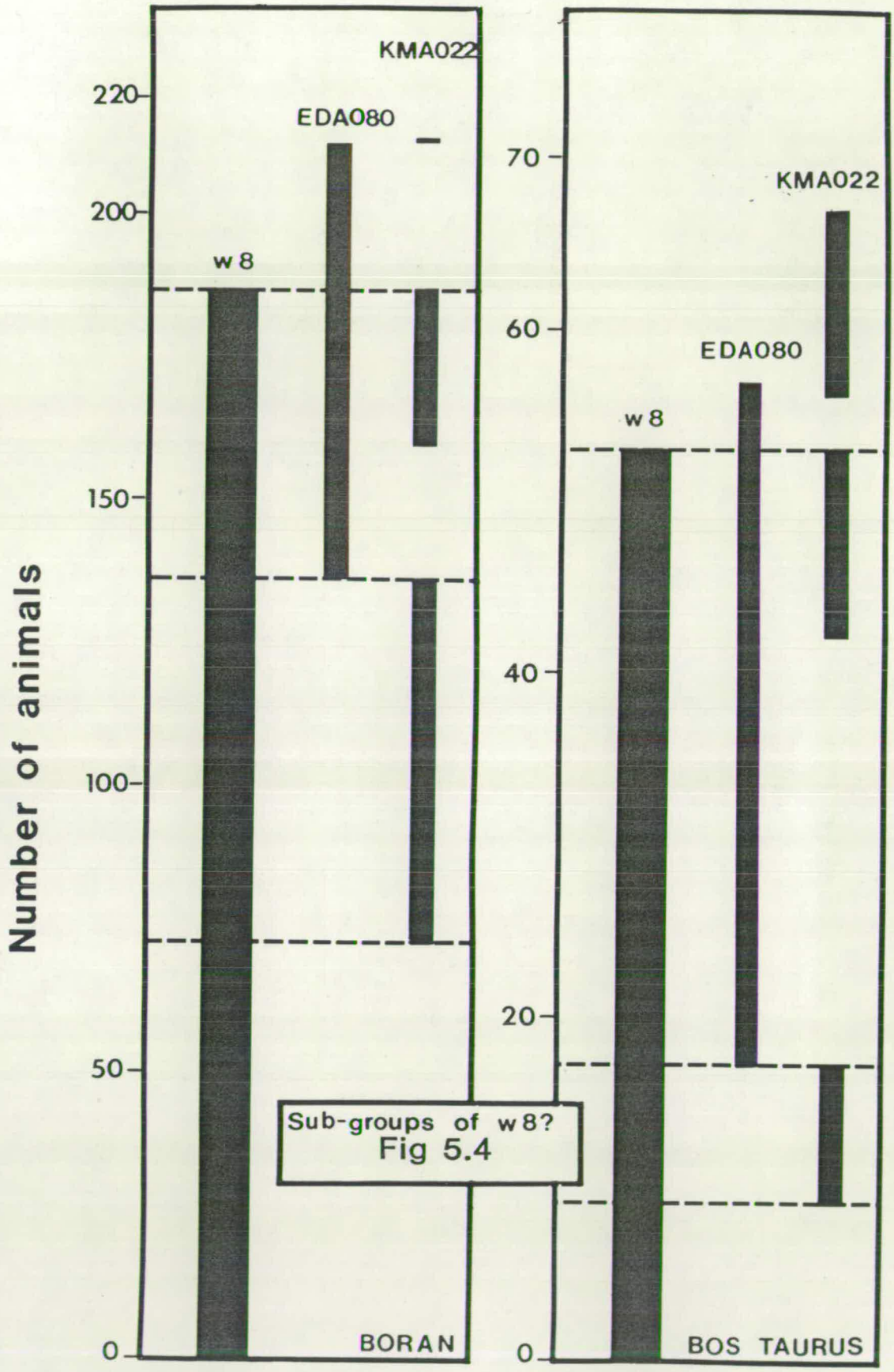
population	SERA S1	SERA S2	NUMBER +VE S1	NUMBER +VE S2	NUMBER +VE S1&S2	SIMTY (%)
Zebu	W8	EDA013	0	62	121	79.6
	W8	EDA063	0	55	121	81.5
	W8	EDA072	34	144	84	48.6
	W8	EDA074	41	151	76	44.2
	W8	EDA096	48	187	71	37.7
	W8	EDA101	21	332	97	35.5
	W8	KMA009	49	150	71	41.6
	W8	KMA022	52	4	66	70.2
	W8	W8+	114	0	4	6.6
	W8	KM22	52	0	66	71.7
Boran	W8	EDA013	0	134	186	73.5
	W8	EDA014	127	62	59	38.4
	W8	EDA028	123	88	63	37.4
	W8	EDA063	0	15	186	96.1
	W8	EDA072	38	177	148	57.9
	W8	EDA074	27	144	159	65.0
	W8	EDA080	134	25	51	39.1
	W8	EDA099	120	63	66	41.9
	W8	EDA101	51	331	135	41.4
	W8	EDA109	55	324	131	40.9
	W8	KMA009	61	95	123	61.2
	W8	KMA022	93	1	92	66.2
	W8	W8+	134	0	51	43.2
	W8	KM22	93	0	92	66.4
European	W8	EDA013	0	14	53	88.3
	W8	EDA063	0	2	53	98.1
	W8	EDA074	1	89	50	52.6
	W8	EDA080	16	4	36	78.3
	W8	EDA105	31	15	22	48.9
	W8	KMA009	26	59	26	38.0
	W8	KMA022	33	11	19	46.3
	W8	W8+	16	0	36	81.8
	W8	KM22	33	0	19	53.5

Table 5.7: w8+

population	SERA S1	SERA S2	NUMBER S1	NUMBER S2	+VE S1&S2	SIMTY (%)
Zebu	W8+	EDA001	0	101	4	7.3
	W8+	EDA013	0	176	4	4.3
	W8+	EDA063	0	169	4	4.5
	W8+	EDA080	0	4	4	66.7
	W8+	EDA101	0	422	4	1.9
	W8+	ED101B	0	104	4	7.1
	W8+	EDA109	0	417	3	1.4
	W8+	W8	0	114	4	6.6
Boran	W8+	EDA013	0	268	51	27.6
	W8+	EDA063	0	149	51	40.6
	W8+	EDA072	3	276	48	25.6
	W8+	EDA080	0	25	51	80.3
	W8+	KMA022	23	65	28	38.9
	W8+	W8	0	134	51	43.2
	W8+	KM22	23	64	28	39.2
European	W8+	EDA013	0	30	36	70.6
	W8+	EDA063	0	18	36	80.0
	W8+	EDA074	1	104	35	40.0
	W8+	EDA080	0	4	36	94.7
	W8+	W8	0	16	36	81.8
	W8+	KM22	24	8	11	40.7

Table 5.8: KM22

population	SERA S1	SERA S2	NUMBER S1	NUMBER S2	+VE S1&S2	SIMTY (%)
Zebu	KM22	EDA013	0	114	66	53.7
	KM22	EDA063	0	107	66	55.2
	KM22	EDA072	2	165	61	42.2
	KM22	EDA074	4	168	58	40.3
	KM22	ED101B	34	78	32	36.4
	KM22	KMA022	0	4	66	97.1
	KM22	W8	0	52	66	71.7
Boran	KM22	EDA013	0	227	92	44.8
	KM22	EDA063	0	108	92	63.0
	KM22	EDA072	4	236	88	42.3
	KM22	EDA074	1	211	91	46.2
	KM22	KMA009	19	145	73	47.1
	KM22	KMA022	0	1	92	99.5
	KM22	W8	0	93	92	66.4
	KM22	W8+	64	23	28	39.2
European	KM22	EDA013	0	47	19	44.7
	KM22	EDA063	0	35	19	52.1
	KM22	EDA074	0	120	18	23.1
	KM22	EDA080	8	28	11	37.9
	KM22	EDA105	6	24	13	46.4
	KM22	KMA022	0	11	19	77.6
	KM22	W8	0	33	19	53.5
	KM22	W8+	8	24	11	40.7


KMA022
EDA080
w 8
220
200
150
100
50
0
Number of animals
Sub-groups of w 8?
Fig 5.4
BORAN
70
60
40
20
0
KMA022
EDA080
w 8
BOS TAURUS

The demonstration of w10 sub-groups

The initial analysis of the activity of the w10 associated sera showed a marked difference between the Bos taurus and the Bos indicus groups. In the Bos taurus animals, w10 was very well defined by EDA069, EDA071 and EDA096. However, in the Bos indicus animals this was not so for the correlation of EDA096 with EDA069 and EDA071 was low. Between EDA069 and EDA071, the similarity index was 50% in the Zebus and 65.6% in the Borans. There was also evidence that two reagents, KKA004 and MA 4.1, were detecting a sub-group of w10, and that the serum KMA003 was detecting another.

It seems that w10 is best defined in all groups by the overlap of EDA069, EDA071 AND EDA096. This was the definition of the cluster which was used in the next stage of clustering. To investigate the subgroups, a cluster called M4 was defined by MA 4.1 and KKA004.

Tables 5.9 and 5.10 show the performance of these clusters. In the African cattle, there was a clear relationship between the cluster 7&10 and w10, but none between w7 and w10. Figure 5.3 summarises this. A proportion of w10 animals showed activity for EDA017 and EDA067. Figure 5.5 summarises the occurrence of the proposed w10 sub-groups.

Both EDA096 and EDA105 showed a great deal of non-w10 activity in the Bos indicus animals, but this activity was minimal in the European cattle.

There was good evidence for the existence of subgroups of w10 in the Bos indicus populations. This is summarised in Figure 5.5. The activity of the Muguga-made serum KMA003 was entirely within w10 in the Bos indicus animals. It reacted with only one animal in the Bos taurus population, and this animal was negative for all w10 defining sera. In the Borans, the activity of the ILRAD-made reagents KKA004 and MA 4.1 was largely confined to w10 animals. These sera were not tested in the Zebu or Bos taurus groups.

The correlation between MA 4.1 and KKA004 was very close, and the one discrepancy could well have been an error. The similarity indices of the cluster M4, which was considered to be detected by these two reagents, is shown in table 5.10. Both reagents were made by immunisation with the same animal (B641, a w10,KM3,M4/KM18 Boran).

Ten N'dama calves from The Gambia were tested with the w10 defining sera. Four of these were w10, and all four also reacted with KMA003 and the M4 defining reagents. Forty nine Friesian/Sahiwal crosses were also tested, 14 of which were w10, and none were M4 or KMA003 positive.

Another monoclonal antibody, MA 7.1, showed activity for all w10 animals and many others in the Borans (the only group in which it was tested). It was made by

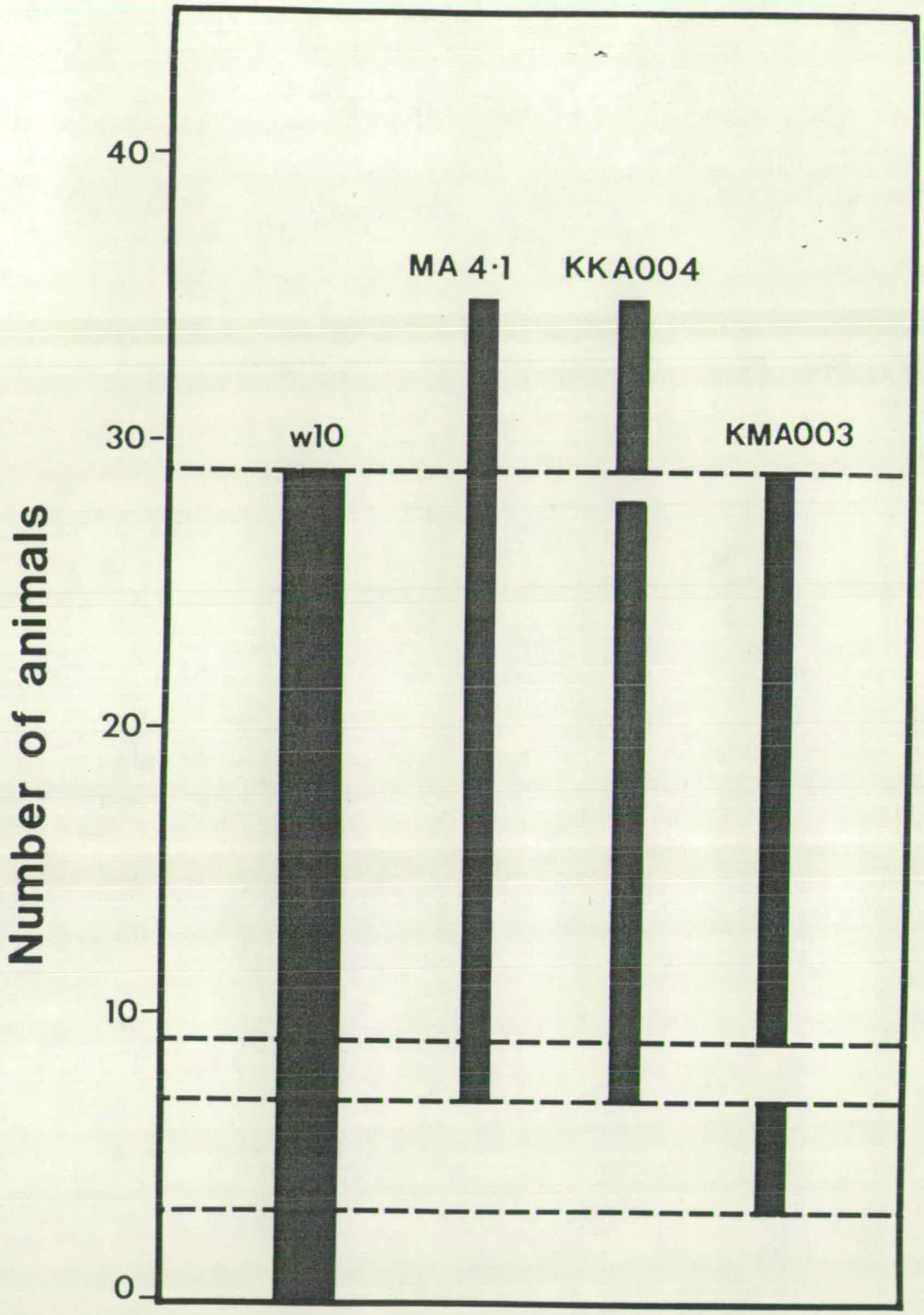
immunisation with lymphocytes from a Friesian (C17; w7/w10). It also had reactivity for all KM17 animals, as well as reacting with many animals which were neither KM17 nor w10. However there is no correlation with any other serum. All proposed clusters (except for w16, seen in only one animal) have animals which are MA 7.1 positive, and more which are MA 7.1 negative.

Table 5.9: w10

population	SERA S1	SERA S2	NUMBER +VE S1	NUMBER +VE S2	NUMBER +VE S1&S2	SIMTY (%)
Zebu	W10	EDA069	0	14	23	76.7
	W10	EDA071	0	38	23	54.8
	W10	EDA085	2	266	21	13.5
	W10	EDA096	0	225	23	17.0
	W10	EDA105	1	226	22	16.2
	W10	KMA003	16	0	7	46.7
	W10	7&10	15	0	8	51.6
Boran	W10	EDA017	12	64	47	55.3
	W10	EDA067	23	100	36	36.9
	W10	EDA069	0	31	59	79.2
	W10	EDA071	0	31	59	79.2
	W10	EDA072	5	271	54	28.1
	W10	EDA085	2	221	56	33.4
	W10	EDA096	0	172	59	40.7
	W10	EDA105	0	311	59	27.5
	W10	KMA003	17	3	42	80.8
	W10	KKA004	2	4	15	83.3
	W10	MA04.1	7	7	21	75.0
	W10	MA07.1	0	131	28	29.9
	W10	7&10	27	0	32	70.3
	W10	M4	3	4	14	80.0
European	W10	EDA069	0	1	56	99.1
	W10	EDA071	0	1	56	99.1
	W10	EDA085	0	55	56	67.1
	W10	EDA096	0	1	56	99.1

Table 5.10: M4

population	SERA S1	SERA S2	NUMBER S1	NUMBER S2	+VE S1&S2	SIMTY (%)
Zebu	M4	NOT TESTED				
Boran	M4	EDA017	5	21	13	50.0
	M4	EDA067	6	36	12	36.4
	M4	EDA069	4	18	14	56.0
	M4	EDA071	3	12	15	66.7
	M4	EDA076	17	0	1	10.5
	M4	EDA085	0	58	17	37.0
	M4	EDA087	12	2	6	46.2
	M4	EDA105	1	69	17	32.7
	M4	KMA003	4	3	14	80.0
	M4	KKA004	0	1	18	97.3
	M4	MA04.1	0	0	18	100.0
	M4	MA07.1	0	70	18	34.0
	M4	7&10	7	3	11	68.7
	M4	W10	4	3	14	80.0
European	M4	NOT TESTED				



Sub-groups of w10 in Borans

Fig 5.5

Discussion

w6

In this study, a striking feature of the results of the initial analysis was the consistency of the relationship between the w6 defining reagents. KMA038 was made in Kenya by a skin implant into a non-w6 Boran from its calf of unknown BoLA type. Its correlation with the Edinburgh w6-defining sera is remarkable.

It is, however, clear that EDA097, which in Europe is an excellent w6-broad defining serum, is detecting at least two specificities, one of which (KM18) is unknown in European animals.

The definition of the workshop sub-groups of w6 was not clear in the African animals, and it may be that their apparent occurrence outside w6 is due to cross-reactivity with antigens unrelated to those which occur as sub-groups of w6 in European animals.

One serum which showed good evidence of specificity for a w6 sub-group was KMA025, which was made by the immunisation of a w6 Friesian with *Theileria parva* infected lymphoblasts from a w6 Friesian. This is the type of immunisation that would be expected to generate an anti - w6-sub-group antiserum. It has not shown activity for any *Bos taurus* animals other than the donor. However, the donor came from a farm where no detailed breeding records were kept, and where *Bos taurus* and *Bos indicus* animals of a wide variety of breeds are allowed to run

together. It is therefore possible that it had some Zebu blood. It seems likely that KMA025 is detecting a w6 sub-group which is extremely rare in Bos indicus animals. Although the fact that the animals with the KM25 specificity were all zebus from Northern Kenya, makes it possible that the reactions of KMA025 were due to a specificity independent of w6 but controlled by a single haplotype common to these animals

In summary, w6 showed evidence that it was itself a split of the antigen detected by EDA097. This had not previously been observed because the other part of this split (KM18) is unknown in Europe. The existing w6 sub-groups w6.1 and w6.2 were very poorly defined in the African cattle, and may not have occurred at all, and there is some evidence for a new and extremely rare sub-group of w6, detected by KMA025.

w8

This study strongly supported the view that w8 is best defined by the sera EDA013 and EDA063, and that EDA080 detects a sub-group. This sub-group is common in Europe, less common in the Borans and rare in Zebus. EDA080 also appears to have some non-w8 activity which is of similar frequency in all groups. A second w8 sub-group is defined by KMA022 and is also of comparable frequency in all populations. It is possible that it detects a sub-group for which the workshops have offered evidence. These sub-groups do occur together (including animals with other putative first locus alleles) so they may be

distinct from the apparently mutually exclusive w6 subgroups, and more akin to the proposed w10 subgroups, KM3 and M4.

In the European animals, approximately 20% (8 of 44) of the animals with reactions in the w8+/KM22 complex had KM22 reactions without w8+. In the Borans, over 55% of such animals had only KM22. In the Zebu animals, only one of the 69 animals of this type had both reactions, while 94% have only KM22. The occurrence of KM22 in animals whose PBL did not react with EDA080 is therefore a case in which a gradation is seen between the European and Zebu animals, with the Borans being of intermediate type. One explanation of this pattern would be the occurrence of a specificity which reacts with both EDA080 and KMA022, so that there are three mutually exclusive sub-groups of w8. Immunisations and absorptions to examine this are under way.

KMA022 does react in non w8 animals at a low frequency (there are 5/606 such animals in the Zebus, 2/662 in the Borans and 11/351 in the European group). It is likely that this is due to extra antibodies and, absorption studies of this serum are required to confirm this.

There can be little doubt however, that this study has demonstrated w8 sub-groups. The reason that they were not easily seen in European cattle is that the large majority of w8 animals contained a single sub-group, that

detected by EDA080. The second sub-group, KM22, does occur in Europe in a fraction of the w8 animals and could have been serologically detected, although apparently it has not yet been found. This is a good illustration of the value of new populations to the serology of BoLA.

w10

There was apparent cross-reactivity between two w7 defining sera (EDA017 and EDA067) and the w10 specificity. This cross-reactivity is seen in both African populations and is completely absent in European cattle. A possible explanation is that these sera react with an antigen associated with w10 in Africa, which is rare in Europe, and which shares determinants with the w7 specificity. This would require that the four w7 defining sera are detecting at least two epitopes on the w7 molecule, one of which occurs on w10 in Africa. Since w7 and w10 are related in that they are both splits of the EDA085 determinant, this explanation does not seem unreasonable and amounts to a subtype of w10 which is unknown in Europe.

There is also good evidence for the existence of two w10 sub-groups in the African cattle, KM3 and M4, both of these often occur together in the same animal and are clearly not allelic. Both appear to be entirely absent in the European cattle. The one reaction of KMA003 with Bos taurus cattle is likely to be either a false positive or a cross-reaction with a completely different antigen,

although further tests to confirm this are underway. MA 4.1 was not tested in European cattle during this study. However, KKA004 has since been tested in Edinburgh, upon 235 cattle of a wide range of Bos taurus breeds. It showed activity for three cattle none of which were w10, although there were 46 w10 animals in the group (Spooner, personal communication).

This study has shown clear evidence of w10 subgroups with a frequency which is very much higher in both African populations than in the European cattle. It would be valuable to compare them with the sub-groups detected by American reagents at both international workshops, and this will be done at the next BoLA comparison test.

The cluster which detects M4 is unique in BoLA-typing in that it is the only example of a monoclonal antibody and an alloantiserum which show almost perfect correlation. This is seen even when they occur outside w10, and makes it highly likely that they are detecting the same epitope. Karr (1984) reports two anti-HLA-class II MoAbs which in population studies appear to detect the same specificity as alloantisera, but in biochemical studies, they precipitate different molecules. However, this is not directly comparable with MA 4.1 and KKA004 in that the structure of class-II molecules is different from that of class I and the association between the M4 defining reagents in this study is very close. It is possible that the non-w10, M4 animals have this epitope on an antigen which is completely distinct from w10, so that

this activity should not be considered as cross-reactive. An alternative explanation is that M4 is the product of another locus, although the fact that its association with w10 persists in West African N'damas makes this unlikely.

The w10 sub-groups have also been investigated with MLC-generated alloreactive CTL (Teale, personal communication), which can be more discriminative than alloantisera (Lopez de Castro et al, 1984). This work has shown that there are CTL which have activity for the w10 specificity of both European and African cattle, irrespective of the presence of KM3 and M4. There is also evidence that some CTL are specific for the M4 antigen, including that on w10 negative M4 targets (Teale, personal communication).

It has also been shown that the serum KKA004 precipitates a 44,000 molecular weight protein, from the lymphocytes of a w10/M4 animal (Bensaid, personal communication).

Chapter 6

THE DESCRIPTION OF PREVIOUSLY UNKNOWN BoLA ALLELES

Introduction

A number of sera raised in Kenya clustered together, and reacted with a small number of animals in such a way as to suggest that they were detecting previously unknown BoLA antigens. This detection of new BoLA antigens was not surprising, for although the internationally agreed specificities did occur in the African cattle, the frequency of some was extremely low and this suggests that there are specificities more common in African than European cattle. Racially characteristic specificities are well known in HLA-typing (Dick, 1978; Terasaki, 1980; Albert *et al*, 1984).

Methods

Initially, new specificities were detected by searching for Kenya-made antisera which clustered together, but independently of the Edinburgh reagents. The method of analysis and the criteria for significance were as described in chapter 5.

Subsequently, single sera were considered as potentially detecting specificities. This is not as reliable as the use of clusters, because many reactions may be due to second or third specificities which clustering with another serum would help to identify. The criteria for a potentially useful single serum were; a relatively rare occurrence; a similarity index with all other clusters and antisera of less than 10%, and no evidence of activity for sub-groups of other clusters.

Results and Discussion

KK1

The serum KKA001 had a 100% similarity index with KKA002 in the one population in which it was tested. The acitivity of these two appeared to be entirely within that of KMA014. Both KKA001 and KKA002 were made by grafts between Boran dam/calf pairs, both the donors being positive for KMA014 while the recipients were negative.

KMA014 has been successfully absorbed in very small quantities with PBL taken from an animal which was KMA014 positive, KKA001 and KKA002 negative. This created a reagent which had no activity for the absorbing cell, but which retained activity for PBL from two animals whose PBL reacted with KKA001 and KKA002. Unfortunately the animal which provided the absorbing cells died before further absorptions could be made. It seems certain, however, that KMA014 contains at least two antibodies, one of which is highly correlated with KKA001 and KKA002.

A cluster was therefore declared called KK1, which comprised these three reagents. Table 6.1 shows the results of the similarity index analysis with this cluster. Only four KK1 animals were found (of 592 which were tested with all three sera) so spurious associations are to be expected. However the cluster appears well defined. The apparent association with four Edinburgh sera could well be due to chance.

Table 6.1: KK1

population	SERA S1	SERA S2	NUMBER +VE S1	NUMBER +VE S2	NUMBER +VE S1&S2	SIMTY (%)
Zebu	KK1	NOT TESTED				
Boran	KK1	EDA065	0	34	4	19.0
	KK1	EDA120	0	19	4	29.6
	KK1	ECA121	0	19	4	29.6
	KK1	EDA125	0	60	4	11.8
	KK1	KMA014	0	15	4	34.8
	KK1	KKA001	0	0	4	100.0
	KK1	KKA002	0	0	4	100.0
European	KK1	NOT TESTED				

KK3

This cluster is based upon the serum KKA003, the activity of which appeared to be almost entirely confined to animals positive for three other sera, EDA089, EDA090 and EDA118. The cluster KK3 was therefore considered to be detected by these four antisera.

Table 6.2 shows the performance of this cluster in the Borans. Ten positive animals were found. KK3 seemed to be a single antigen, and was well defined by KKA003 and EDA090.

Table 6.2: KK3

population	SERA S1	SERA S2	NUMBER +VE S1	NUMBER +VE S2	NUMBER +VE S1&S2	SIMTY (%)
Zebu	KK3	NOT TESTED				
Boran	KK3	EDA089	0	34	10	37.0
	KK3	EDA090	0	2	10	90.9
	KK3	EDA118	0	26	10	43.5
	KK3	KKA003	0	9	10	69.0
European	KK3	NOT TESTED				

KM30

Analysis of the activity of the serum KMA030 showed that it had a similar relationship with other antisera (but not a similar reaction frequency) in all three populations. It appeared to be closely related to EDA030, and reacted only with animals positive for EDA091. The reactions of KMA011 were confined to animals which also reacted with KMA030. The cluster KM30 was defined by the overlap of these four reagents and table 6.3 shows their performance. There was good evidence for a single antigen detected by this group of sera.

Three of these sera, EDA091, KMA030 and KMA011, were assigned to the specificity EU1 by the European workshop, where EU1 appeared to include w1, w3, w7, w11 and w12. This is clearly not the case with KM30, although EDA091 does seem to have some association with w11 and w16. The reasons for this difference of interpretation are not clear. EU1 was a poorly defined cluster, and the effect may have been due to the small sample size used at the workshop. The performance of the cluster KM30 was comparable in all populations.

Table 6.3: KM30

population	SERA S1	SERA S2	NUMBER S1	NUMBER S2	+VE S1&S2	SIMTY (%)
Zebu	KM30	EDA030	0	127	96	60.2
	KM30	EDA091	0	141	96	57.7
	KM30	KMA011	0	22	96	89.7
	KM30	KMA030	0	91	96	67.8
Boran	KM30	EDA030	0	98	55	52.9
	KM30	EDA091	0	128	55	46.2
	KM30	KMA011	0	18	55	85.9
	KM30	KMA030	0	56	55	66.3
European	KM30	EDA030	0	47	24	50.5
	KM30	EDA091	0	118	24	28.9
	KM30	KMA011	0	15	24	76.2
	KM30	KMA030	0	49	24	49.5

KM17

Following initial similarity index analysis, the Kenyan serum KMA017 appeared to cluster with another Kenya-made serum, KMA029. Cluster KM17 was declared, which was considered to be detected by KMA017 and KMA029.

Table 6.4 shows the result of this analysis. The association between the two sera was high, KMA017 having more positive reactions than KMA029. The frequency of the specificity was comparable in the two Bos indicus populations, but KM17 was entirely absent in the European cattle.

In both African populations KM17 was entirely confined to animals positive for EDA105. In the Borans, it was almost entirely confined to animals also positive for EDA072. The monoclonal antibody MA 7.1 was also positive for all KM17 animals, as well as for many non-KM17 animals.

Table 6.4: KM17

population	SERA S1	SERA S2	NUMBER S1	NUMBER S2	+VE S1&S2	SIMTY (%)
Zebu	KM17	EDA105	0	235	21	15.2
	KM17	KMA017	0	23	21	64.6
	KM17	KMA029	0	6	21	87.5
Boran	KM17	EDA072	1	290	29	16.6
	KM17	EDA105	0	329	30	15.4
	KM17	KMA017	0	37	30	61.9
	KM17	KMA029	0	0	30	100.0
	KM17	MA07.1	0	144	15	17.2
European	KM17	DOES NOT OCCUR				

KM4

Reactions with the serum KMA024 were not common in either of the Bos indicus groups, and in the Bos taurus population it reacted with only two animals. It had a high correlation with KMA004, which reacted with some KMA024 negative animals. Despite the extremely low number of animals which reacted with this pair of sera, there was no evidence of association with any other sera or clusters. This alone was evidence that they were detecting a BoLA allele. KMA024 and KMA004 were therefore assigned to the specificity KM4.

The results of analysis with this cluster are shown in table 6.5. The correlation between the two was excellent, and it seems safe to assume that they were detecting a single antigen.

Table 6.5: KM4

population	SERA S1	SERA S2	NUMBER +VE S1	NUMBER +VE S2	NUMBER +VE S1&S2	SIMTY (%)
Zebu	KM4	KMA004	0	4	9	81.8
	KM4	KMA024	0	0	9	100.0
Boran	KM4	KMA004	0	2	5	83.3
	KM4	KMA024	0	1	5	90.9
European	KM4	ONLY ONE ANIMAL POSITIVE				

KM18

The reactions of the two Kenyan sera, KMA010 and KMA018, appeared to cluster together. They correlated well in the two Bos indicus populations, KMA010 having activity for some animals which did not react with KMA018. In the Borans, they also showed associations with several other reagents; EDA113, EDA114, EDA115, EDA093 and MA P3. Unfortunately, none of these sera were tested in the Zebu group. EDA097, a w6 defining serum, was positive for all KM18 animals as well as w6 animals (Figure 5.2). EDA115 and MA P3 also reacted with all KM18 animals, and many others.

In the Bos taurus group, the situation was very different. Reactions of KMA010 were not uncommon, but EDA018 occurred in only three animals. The relationship with EDA093 appeared to remain, and suggests that this serum is detecting the non-KM18 element of KMA010. EDA113 was more closely related to KMA018 than to KMA010, and all three KMA018 reacting cells were EDA113 positive.

EDA093 is a w6.1 defining antiserum. At the European workshop, KMA010 was assigned to w6.1, having complete correlation with this cluster. It seems that this activity is shown by KMA010 only in European animals, and that it is distinct from the cluster defined by the two sera KMA010 and KMA018.

In an attempt to unravel this complex, KM18 was first defined as KMA018, KMA010, EDA097 and EDA093. Table 6.6

shows the results of this clustering. Clearly, the cluster KM18 was extremely rare in Europe. The reactions of MA P3 were all with KM18 animals. EDA093 correlated well with KM18 in the African animals, and not at all with it in Europe. There were 53 EDA093 reactions in Europe, and the similarity between this serum and KMA010 was 41.1%. It seems that KMA010 has an anti-w6.1 component, and that this is distinct from the KM18 component. Thus in European cattle, where there are few KM18 animals, KMA010 appears to be a good w6.1 antiserum, and it appears to be a good KM18 antiserum in Africa where there are few w6.1 animals.

That KM18 is a BoLA product is supported by the use of primary-MLC-generated alloreactive CTL, which have activity only for cells with the KM18 specificity (Teale, personal communication). It has also been shown that the MoAb MA P3 precipitates a typical class I molecule, of approximately 44,000 daltons moecular weight (Bensaid, personal communication)

Table 6.6: KM18

population	SERA S1	SERA S2	NUMBER +VE S1	NUMBER +VE S2	NUMBER +VE S1&S2	SIMTY (%)
Zebu	KM18	EDA092	12	19	10	39.2
	KM18	EDA093	0	24	23	65.7
	KM18	EDA097	0	113	23	28.9
	KM18	KMA010	0	24	23	65.7
	KM18	KMA018	0	3	23	93.9
Boran	KM18	EDA093	0	10	16	76.2
	KM18	EDA097	0	176	16	15.4
	KM18	EDA113	0	70	12	25.5
	KM18	EDA114	4	4	8	66.7
	KM18	EDA115	0	31	12	43.6
	KM18	KMA010	0	22	16	59.3
	KM18	KMA018	0	15	16	68.1
	KM18	MA05.1	0	33	12	42.1
	KM18	MA P3	0	5	6	70.6
European	KM18	OCCURS IN ONLY TWO ANIMALS				

KM33

In the European animals, reactions of EDA040 were seen in 14 animals. These reactions were largely confined to animals which also reacted with the serum EDA124.

However, in the Zebus EDA040 reactions were common, 88 animals being positive for this serum. EDA124 was not tested in this group. In the Borans, EDA040 reactions were rarer, nine animals being positive. There was no evidence of an association with EDA124 in these animals.

However, both of the Bos indicus groups showed signs of a specificity entirely confined to animals positive for EDA040. The Kenyan serum KMA033 reacted with 21 of the 88 EDA040 positive Zebus, and with only one EDA040 negative animal. In the Borans, this serum reacted with one of the nine EDA040 positive animals, and with only two others. KMA033 has never shown a reaction in Bos taurus animals.

The European serum EDA040 was assigned by the European workshop to the specificity EU12, which appeared to be a BoLA-A locus product. It is clear from this study that EDA040 contains at least two specificities, including one which is rare in Europe. It is surprising that such a population of antibodies could be raised in a population which appears not to contain the KM33 specificity. One explanation is that the two specificities, EU12 and KM33 are controlled by completely different alleles but that the determinant detected by EDA040 is common to the products of both.

There can be little doubt that the African animals which reacted with both EDA040 and KMA033 did not express the EU12 molecule. It is possible that the EU12 molecule was present in the animals which were KMA033 negative. There is therefore good evidence here of a specificity, KM33, which is rare or unknown in European cattle and not uncommon in Bos indicus. The frequency of the KM33 specificity was another case in which the Borans seemed to be intermediate between the European animals and the Zebus.

Single serum defined specificities

Apart from the sera described above which formed clusters, a number of sera which were made in Kenya showed no association with any other sera or clusters of sera. The results of the initial similarity index calculations clearly showed these reagents and the frequency of their reactions in the different populations.

Examination of these results suggested that the following sera might be detecting products of a single locus, because they had no significant associations with other antisera and because they reacted with lymphocytes from a small fraction of animals; KMA001, KMA008, KMA012, KMA021, KMA023, KMA027, KMA028, KMA039, KMA040. The frequency of the reactions of these sera is shown in Table 6.7

Table 6.7

Frequency (%) of animals reacting with some Kenyan sera

	% FREQUENCY OF POSITIVE ANIMALS		
SERUM	ZEBU	BORAN	EUROPEAN
KMA001	3.49	1.84	3.29
KMA008	14.40	12.15	0.58
KMA012	2.67	13.08	0.58
KMA021	2.01	0.46	0.87
KMA023	1.50	1.69	2.05
KMA027	0.83	0.31	0.58
KMA028	1.18	0.77	4.97
KMA039	1.03	1.40	0.00
KMA040	1.55	0.93	0.58

Conclusions

There was good evidence of several new specificities, although only those detected by clusters of reagents were well defined. Those defined by a single serum can only be considered tentative because the sera are unlikely to be monospecific. However, two of the most frequent of these, KM8 and KM12, have since been shown to cluster with other sera made independently, and appear to detect single specificities extremely reliably (Kemp and Teale, unpublished observations).

Chapter 7

THE GENETIC CONTROL OF THE NEW SPECIFICITIES

Introduction

The panel of antisera used in this study included sera which detect all of the internationally agreed BoLA specificities. Differences in the performance of these sera were expected when they were used in a new population such as the African cattle, since apparent losses of specificity are well known in the HLA field when sera are used in different populations (Dick, 1978). It could not therefore be assumed that, when all the sera of a cluster reacted with an animal, that the animal was positive for the antigen detected by that cluster in European cattle. The first task therefore in examining the results of the BoLA-typing, was to consider the activity of these well defined European reagents and to decide how far their operational monospecificity was changed in the African cattle. Having done this, it becomes possible to consider the genetic control of the new specificities which this study has proposed.

Methods

The similarity index programme provided a great deal of information about the way the defining sera were behaving. It was expected that a loss of specificity would not affect all sera of a cluster in the same way, so that cross-reactivity of the sera would be revealed by a reduction in the similarity between the members of a

cluster. Such a reduction was seen in all clusters except w6.

A useful way to determine whether or not antigens are controlled by the BoLA-A locus is to look for "triples", that is antigens which occur with two or more other BoLA-A locus products. If this happens, it is certain that either one of the antigens is not an A locus product, or that two clusters are detecting the same antigen. Therefore the selection and printout programmes were used to examine the way in which the clusters occurred. It may be significant that the similarity index analysis did not reveal any clusters which were associated with each other, except those considered to be subgroups.

Results

An initial examination of the internationally agreed clusters showed that some made triples, most of which involved w3, w4, w8 without its subgroups, w9 and w20. Triples were more common in the African animals than the European ones.

w3 formed two triples (in addition to those caused by reactions with the w9, w20 and w8 sera) both of which were in the Borans. One of these animals was w3/w6/w13, and the other was w3/w7/w13. Since w3 is defined by a single serum (EDA039), it is not surprising that it occasionally formed triples. In Europe, all w3 animals are positive for EDA001 (Spooner *et al*, 1979a; Anon, 1982). Both of the African w3 triples were negative for EDA001 and this

demonstrated that in the African cattle EDA039 was detecting a non-w3 antigen, and that the true w3 antigen occurred only in EDA001 and EDA039 positive animals.

The w4 defining serum (EDA082) made four triples, all in the Borans. Like the w3 reagent, this single serum seemed to detect a BoLA-A locus product and to have some cross-reactivity with other antigens. EDA117 has a high similarity index with the w4-defining serum in all populations. If the definition of w4 was restricted to those animals which were also positive for EDA117 then no triples were seen. Unfortunately, this serum was not tested in the Zebus, and w4 had therefore to be considered undetected in this population.

w8 showed six triples in the Borans, eight in the Zebus and none in the European population. Of these 14 triples, only six involved w3 or w4. It seems that in both African populations w8, as defined by EDA013 and EDA063, was detecting an extra antigen which was distinct from the European w8. If the definition of w8 was limited to those animals also showing EDA080 reactions or KMA022 reactions (i.e. w8+ or KM22), then there were only three triples, all in Borans, and two of which involved w4 animals which did not react with EDA017. This suggested that it was reasonable to define two antigens, w8+ and KM22, which appear to be the products of BoLA-A alleles.

However, KM22 and w8+ are not allelic to each other. In all populations there are many w8+/KM22 animals with

other first locus alleles. The best explanation seems to be that there is a basic antigen defined by w8 which has sub-groups defined by w8+ and KM22. In Europe, w8 showed no triples, but in Africa it was impossible to define this antigen because of non-w8 reactions in the defining sera EDA013 and EDA063.

In both Borans and Zebus, w9 showed many triples which included almost every other cluster. In the European animals, w9 was much less common and showed no triples. Clearly the w9 sera detected more than one antigen in the African cattle. Similarly, w20 showed many triples in both African populations. In the Borans, there were five animals with triples including w20.

In view of this, w3 was re-defined as requiring reactions of EDA039 and EDA001. This meant that if an animal was w1/w3 it was impossible to know if it was a genuine w3, because EDA001 is also a w1 defining serum. w4 was re-defined as occurring only in EDA082 and EDA117 positive animals. w8 was discarded as a useful cluster, so that only w8+ and KM22 were considered to be BOLA-A locus alleles. The definition of w4, w9 and w20 was considered to be inadequate for further analysis.

None of the other internationally recognised clusters showed any triples. Some, of course, are extremely rare in the African cattle, and so triples would not be expected even if they were detecting more than one BoLA-A product or a non-BoLA-A product.

Having shown that, with the exceptions noted above, the internationally agreed clusters perform as products of alleles at the same locus, it was then possible to examine the way in which the unassigned clusters and sera performed. The reactivity of these sera was studied in relation to the known clusters described above. No attempt was made to compare their relationship with each other until this had been done.

Table 7.1 shows the number of triples shown by each of these Kenyan sera, independently of the others, in relation to their frequency and the frequency of animals with two "good" workshop clusters (i.e. excluding w4, w9 and w20 and restricting the definition of w8 to w8+ and KM22 and that of w3 to w3 with EDA001 reactions) in each population.

Table 7.1 - Observed and expected numbers of triples

Expected number of triples were calculated by multipling the frequency of the antigens detected by the sera by the frequency of "good doubles" in a population. If these specificities were assorting independently of the workshop specificities, then this is the number of triples which would be expected. A number lower than this suggests that, at least part of the activity of a serum is directed against a specificity which is an allele of the BoLA-A locus.

	Expected number of triples if non-allelic to BoLA-A			Observed number of triples			
	Boran	Zebu	European	Boran	Zebu	European	Therefore: Allelic ?
KM17	4	1	0	0	0	0	Yes
KM4	1	0	0	0	0	0	?
KM18	2	2	0	1 $	1 &	0	Yes
KMA001	2	1	3	0	1	4	No
KMA008	11	6	1	3	1	0	Yes *
KMA012	11	1	1	0	0	0	Yes
KMA021	0	1	1	0	0	0	?
KMA023	1	1	2	0	0	2	?
KMA027	0	0	1	0	0	0	?
KMA028	1	0	5	1	0	3	No
KMA033	0	1	0	0	0	0	?

Observed number of "good" doubles: Boran 87/665 = 13.1%
Zebu 40/606 = 6.6%
European 99/351 = 28.2%

$ Due to a w3/w1 animal, w3 cannot be identified in this situation.

& Due to a w6.1 animal.

* three of the four triples are due to w10 without KMA003 or KK4.

Discussion

The cluster KK1, which was only tested for in the Borans, occurred in four animals, none of which formed triples. However, four animals is not enough to judge the significance of this result.

KK3 was seen in ten Borans, and one of these formed a triple of the type w5/KM22/KK3. Again, it is not possible to draw many conclusions.

KM17, which was seen in 30 Borans and 21 Zebus, did not form any triples. This cluster did not occur in any European animals. It seems likely from these results that KM17 is the product of a BoLA-A locus allele.

KM4 occurred in five Borans, nine Zebus and one European animal, and did not form triples in any of these animals.

KM18 occurred in 16 Borans. There was one possible triple in this group, an animal which was w1/w3/KM18. However, since the animal is w1 and therefore reacts with EDA001, it is likely that it is not a true w3. In the Zebus, KM18 occurred in 23 animals where it formed one triple of the type KM22/w6.1/KM18. There is an association between one of the KM18 defining sera (KMA010) and w6.1, and it is therefore possible that this was not a true KM18 animal. The evidence seems good that the KM18 defining sera are detecting the product of an allele at the BoLA-A locus, except where w6.1 is present when cross-

reactivity may occur.

The serum KMA001 reacted with a comparable proportion of animals in all populations. It created many triples in all. In the European animals triples were seen with an apparently random mixture of clusters, and in both African populations KMA001 positive animals were frequently positive for the w9 defining serum. The similarity index calculations suggested that this serum was associated with w9 in both Zebus and Borans, the similarity indices being 16.8% and 22.4% respectively. In both populations the w9-detecting serum showed activity for more animals than did KMA001, although there were some animals which were KMA001 positive and w9 negative.

One possible explanation of this situation is that KMA001 is detecting the product of a second BoLA locus. The w9 defining serum is known to show non-w9 activity in the African cattle, and it may be that KMA001 cross-reacts with the antigens responsible for these reactions. It is certain, however, that KMA001 is not detecting the product of a single BoLA-A allele. Whether its pattern of reactivity is due to activity for a range of BoLA-A products, or whether it is due to activity for the products of a small number of alleles controlled by a separate locus, cannot be determined without further study.

There was a significant proportion of animals positive for KMA008 in both African populations, but its

activity was less common in Europe. A large number of triples would be expected to occur if this serum was detecting an antigen which is not allelic to the workshop specificities. In fact, there was only one in the Zebus and three in the Borans. It may be significant that all of these triples involved w10. Of these four w10 triples, three were negative for KM3 and KK4. The fourth involved the rather weak cluster w13. This raises the possibility that EDA069 and EDA071 may be poor at defining w10, and that in fact no true Boran w10 is negative for both KM3 and KK4, or at least that the definition of w10 without these clusters is unreliable, this would be analogous to the w8 situation.

Thus, there was excellent evidence that KMA008 was detecting the product of an allele of the BoLA-A locus. This serum may, however, contain a second antibody population which cross reacts with a w10-associated antigen.

That the determinant detected by KMA008 was indeed a BoLA-A locus product was confirmed by family studies. The Boran Bull 54 is of BoLA-A phenotype w6/KM8. It has eight offspring which, together with their dams, were BoLA-typed in order to examine the segregation of the proposed KM8 specificity. The results are shown in table 7.2. Clearly, the specificity detected by KMA008 is allelic to the bull's w6 allele. The KM8 determinant was passed to

Table 7.2: The inheritance of KM8

The offspring of the w6/KM8 bull, number 54, showing the inheritance of the proposed KM8 determinant as a BoLA-A locus product.

Sire	Dam	Calf
w6/KM8	w6/w8,KM22	w6/w8,KM22
..	-/-	w6/-
..	-/-	w6/-
..	w6/w7	w6/w7
..	w6/-	KM8/w6
..	w6/-	KM8/-
..	w6/-	KM8/-
..	w8,KM22/-	KM8/-

The KM8 determinant was passed to four of eight calves. In only one case is it impossible to exclude the possibility that both w6 and KM8 were inherited from the sire.

four calves, only one of which was also w6, while the dam of this animal was of BoLA type w6/-. The bull appeared to pass w6 to the remaining four calves.

The serum KMA012 reacted with a large number of Borans, fewer Zebus, and only two European animals. Despite the frequency of its reactions, it formed no triples. It seems likely therefore, that at least in the Borans, this serum is detecting the products of a BoLA-A locus allele.

KMA021 reacted in very few animals, three Borans, three European animals and 12 Zebus. It formed no triples. It is not easy to draw any conclusions from these very limited observations. This serum may detect the product of a BoLA-A allele, or an antigen controlled elsewhere.

Reactions of KMA023 were not common in any population. It reacted with 11 Borans, nine Zebus and seven European animals. Two triples were seen in the European cattle. It is not possible to draw any conclusions from these results at this stage.

The reactivity of KMA027 was extremely rare in all groups. It reacted with two Borans, five Zebus and two European animals. No triples were seen.

KMA028 is another serum with activity for a small number of animals. It detected five Borans, seven Zebus and 17 European animals. Among the African animals there

was one triple, while in the European animals there were three, all involving "good" BoLA-A locus clusters. It seems likely that this serum is operationally monospecific in the African animals in view of its low reaction frequency. It is possible that the greater number of reactions in the European cattle was due to extra activity for antigens not seen in African cattle. However, it is certainly possible that this serum was detecting an antigen which is not controlled by the BoLA-A locus. When suitable animals are identified, family studies will be made to investigate this possibility.

The activity of KMA033 was entirely confined to animals which also reacted with EDA040, the EU12 defining serum. There were no KMA033 reacting animals in the European group. There were 21 Zebus which reacted with KMA033, and only one Boran (another example of Borans showing a greater resemblance to European animals than Zebus). There were no triples with workshop clusters, but KM33 formed many triples with an apparently random assortment of proposed BoLA-A specificities detected by Kenya made sera. Since the workshop specificities are not common in this population, and since the proposed specificities hold up very well in every other respect in every other population, it would appear that KMA033 is detecting an antigen which is not controlled by the BoLA-A locus.

It is likely that the antigen detected by KMA033 is completely different from that detected by EDA040 and

is not a sub-group of it.

As a result of this series of analyses the following tentative antigens were proposed.

BoLA-A Products	BoLA-B Products (or C or D....)	Unknown control
w1	KM1	KM21
w2	KM23	KM27
w3	KM33	KM28
w4		KM30
w5		KK1
w6		KK2
w7		
w8+,KM22		
w10		
w11		
w12		
w13		
w16		
KM4		
KM17		
KM18		
KM8		
KM12		

If this scheme is applied to the results of the BoLA typings, the Zebus show 92 animals with two detectable BoLA-A locus products. In addition they show four triples, one triple included w6.1 and KM18, which may cross-react, and and one involves w10 without KM3.

In the Borans there are 184 animals with two detectable A locus alleles. There are also seven triples, of which two are attributable to w10 without KM3 or KK4. In the European animals there are no triples.

The proposed non BoLA-A specificities are too rare to allow any conclusions. No animals form "second locus triples", but only one has two second locus specificities.

The evidence is extremely good that the proposed BoLA-A locus specificities are actually controlled by this locus. It may well be that the defining sera will show extra activity in other populations, as did many of the Edinburgh sera which had been exhaustively tested in Europe.

Much less may be said of the other specificities. KK1 and KK3 appear to be highly specific, in that they react with very few cattle. There is only one triple in the Borans involving these clusters, a w5/KM22/KK1 animal. There is, of course, no reason to believe that the non BoLA-A specificities are controlled by the same locus, or even that they are controlled by loci linked to the BoLA-A locus.

The population distribution of the BoLA alleles

The gene frequency programme was used to calculate the gene frequencies of the proposed BoLA-A alleles in each population. The graph Figure 7.1 summarises the results. All of the Zebu groups were pooled to create the Zebu gene frequencies.

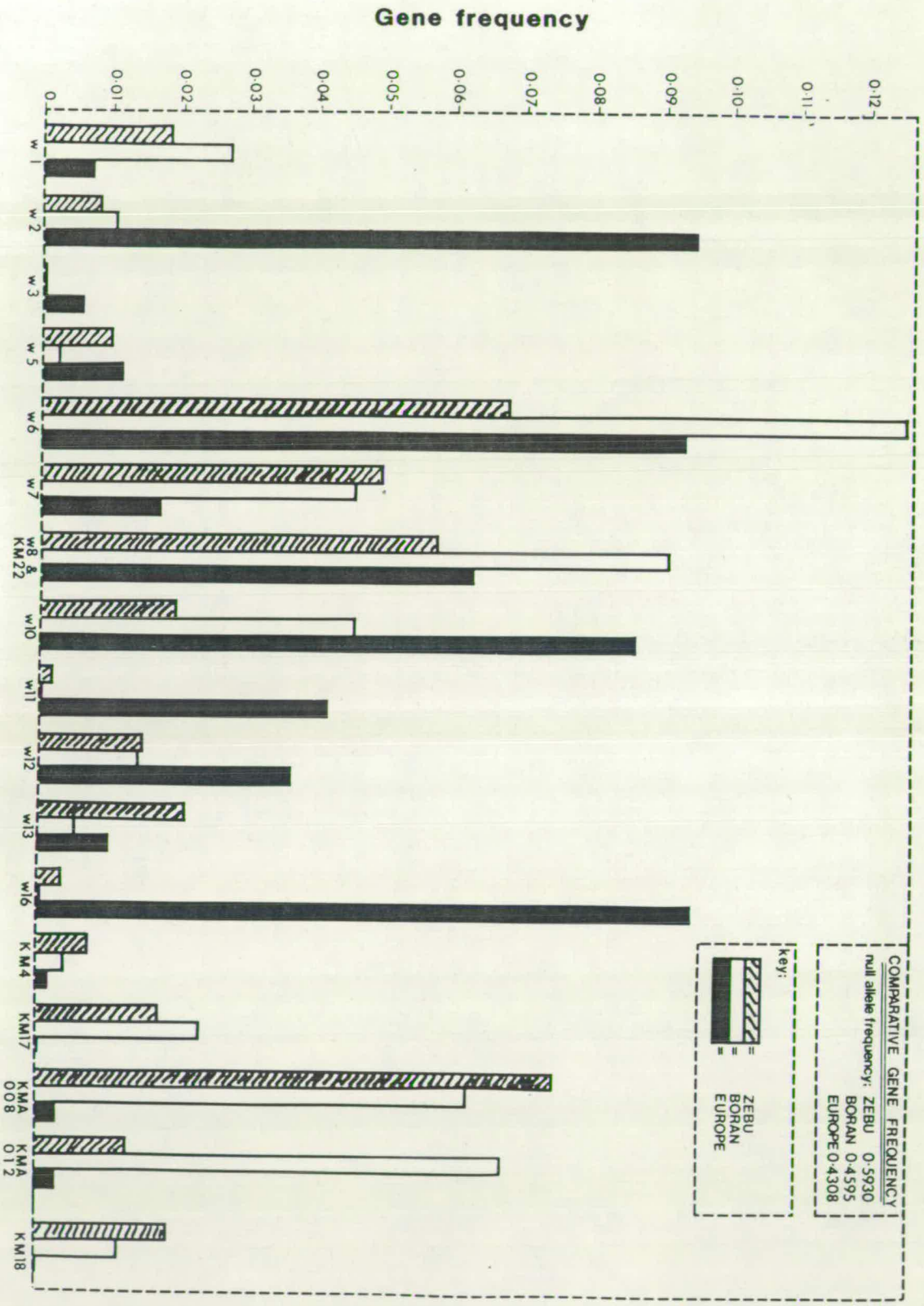


Fig 7.1 Comparative gene frequencies

Chapter 8

DISCUSSION

BoLA Definition

A dramatic and consistent feature of the performance of the BoLA-typing antisera was their loss of specificity when used outside the population in which they were raised and tested. This is not surprising, and was clearly seen in the early days of the study of the HLA system (Dausset and Colombani, 1972). It does, however, suggest that there are a large number of antigens in both populations which are yet to be described. Such differences in the frequency of specificities in different racial groups are well known in HLA (Dick, 1978).

It does not follow that the BoLA product which causes the appearance of these extra reactions in the new population, was present in the immunising animal. It merely suggests that two different products share a common epitope. The sharing of epitopes between products is a common feature of HLA-A and B antigens, and Lopez de Castro *et al* (1984) suggest that a given HLA class I antigen contains eight regions of variability which may correspond to antigenic sites. If this is so, then epitopes shared between different allelic products would not be surprising.

However, the appearance of extra activity when sera are tested in a new group of animals, does imply the

existence of MHC alleles which are undetected in the population in which the serum was raised and tested. The sharing of epitopes further suggests an evolutionary relationship between these new BoLA products, and antigens present in the original population.

Further evidence of differences between the MHC make-up of the African and European groups was provided by the detectable BoLA-A locus alleles. Some were seen in both populations at very different frequencies, while others (w3, w11, w16, KM17, KM18) were extremely rare or undetected in one or other group. Differences in MHC gene frequency is not surprising in view of the number of generations for which these populations have been separated (Epstein, 1971). However, the fact that some specificities, notably w6 and w8, are present in widely diverse populations at similar frequencies while others are virtually unique to one or other, is evidence that BoLA antigens are exposed to selection.

Of course, one crucial question in considering frequencies of BoLA antigens is whether or not the same products are being detected in different populations. The loss of specificity shown by many of the antisera makes it possible that this is not the case. However, many of the clusters comprise four or more sera, and in only one case was there a consistent "shift" of activity in more than one of these defining sera (EDA017 and EDA067 occurring without w7). The overlap of several defining sera at a useful frequency indicates that the specificity detected

can be safely considered to be the same in all groups. There may be differences at a site which is not recognised by any sera of a cluster, but the probability of this decreases with the number of defining sera.

New BoLA alleles

This study provided evidence for the existence of five new BoLA-A locus alleles, which have been designated KM4, KM17, KM18, KM8 and KM12. There is particular clear evidence that two of these, KM8 and KM12, are indeed A locus products. Both occur in African cattle at a high frequency (12% and 13% respectively in Borans). New sera have recently been made which cluster extremely closely with the sera defining these specificities (Kemp and Teale, unpublished observations). The definition of the other three specificities is slightly less secure, although there seems little doubt that they do represent BoLA products. However, their low frequency makes their genetic control hard to determine by population studies, and reduces the confidence which can be placed in the similarity of the defining reagents.

The identification of new and characteristically African specificities was one of the major objects of this study, and KM8 and KM12 alone account for 25% of the BoLA-A locus products in Borans. Only w6 and w8 occur at a higher frequency, and these were both shown to be supertypic specificities. Their apparent occurrence in European cattle is so rare as to make it possible that

they do not truly occur (both KMA008 and KMA012 reacted with approximately 0.6% of European cattle), but may be due to cross-reactivity in the defining sera which was undetected in Africa. It is important to improve the serological definition of these antigens and to examine their occurrence in Europe, and this work is currently under way.

Further class I loci ?

This study failed to provide solid evidence for alleles controlled by a second BoLA locus. When one locus is as well characterised as the BoLA-A locus, it is inevitable that the genetic control of new alleles will be more readily seen when they are also under A locus control. This is because allelism is easy to recognise when a useful fraction of the allelic products are already known.

However, there were no clusters of sera which were clearly not under A locus control. The only potential candidates for B locus control (KM1, KM23 and KM33) were defined by single sera. This suggests that the BoLA-B locus, if it exists, may have poorly antigenic products. There is no evidence of such differential antigenicity in any other species with polymorphic class I genes. The failure of the international BoLA community to demonstrate a second class I locus does however seem to be becoming significant. The distinction of HLA-A and -B was recognised early in the study of the human MHC, and the

definition of the C locus followed soon after that (Bodmer, 1978). The evidence that the BoLA system is different in this respect from the HLA system is mounting.

It is possible that the cow has other class I loci, but that they are monomorphic. In man, the polymorphism of two of the class I loci is high, while the third (HLA-C) has only eight alleles so far recognised (Albert et al, 1984). There are species with monomorphic class I loci, including those with one polymorphic and one monomorphic locus (Streilein et al, 1984). If this were the case in cattle, then the proposed non-BoLA-A defining reagents might be detecting further splits of A locus products.

Another possible explanation would be that the two loci have extremely low recombination frequencies, and so are not separable serologically. If this were the case, then a study such as this one would be expected to detect splits in existing clusters, because it is most unlikely that no recombinations have occurred since the separation of the European and African populations and their exposure to widely different environments. With one exception which will be discussed below, this was not the case. If some of the present clusters are due to the products of two loci then the linkage disequilibrium between them must be very high and must be identical in widely different populations.

Studies based purely on serology, such as this one and that of Stear et al (1982b), cannot be expected to

provide definitive evidence for multiple loci. These workers reported the use of low titre antisera. However, such sera have been shown to be extremely unreliable perhaps because of anti-complement activity (unpublished observations). Also, Stear et al, formed clusters based on sera with correlation coefficients as low as 0.212, which is far below normally accepted levels (de Weck et al, 1976). The results reported here provide considerably less evidence for further BoLA class I loci than those of Stear et al, and this may well be because of the high standard of sera which were initially used to define the internationally recognised specificities. These sera were exhaustively tested and refined at a series of international comparison tests, and they still showed polyspecificity when used in African cattle. Clearly the existence of triples cannot reliably demonstrate multiple loci.

Biochemical studies of the molecules precipitated by the antisera, using an approach such as that described by Hoang-Xuan et al (1982a; 1982b), are capable of demonstrating multiple class I products, given suitable antibodies. Genetic studies using large families of full or half sibs to detect recombinations are another promising means of investigating the genetic nature of the BoLA class I products. Both of these approaches are beginning, using the sera developed during this study.

Sub-groups

The sub-group relationship of some of the specificities described in this study could be explained by the existence of a second BoLA class I locus. One example is the apparent cross-reactivity with w10 shown by two of the w7 defining sera (EDA017 and EDA067). w7 and w10 are serologically related, in that together with w4, they only occur in animals which are also positive for the operationally monospecific serum EDA085. One possibility is that w7 is, in reality, two products controlled by separate loci but occurring together, and that in Africa there has been a recombination giving a product in linkage disequilibrium with w10. However, the facts that these two sera never reacted with animals which were not either w7 or w10, and that the same phenomenon is seen in both *Bos indicus* groups, makes this explanation unlikely. It seems more probable that in the African cattle there is a w10 sub-group which does not occur in Europe. It would not be surprising to find cross-reactivity between previously unknown w10 determinants and the related w7 antigen.

There is also evidence that the sub-groups of w6 occur outside w6 more often in the African cattle than in the European breeds. Within or without, they certainly occur at a very much lower frequency despite comparable frequencies of w6-broad. It may be that these specificities are indeed controlled by a second locus, and that they are in high linkage disequilibrium with w6 in

European cattle, and a lower disequilibrium in African cattle. Such differences have been described in human populations (Bodmer and Bodmer, 1978). This interpretation is supported by the fact that the sub-groups do occur outside w6 in European cattle, albeit at a very low frequency.

However, the w6 sub-groups appear to have survived very well the rigours of international comparison tests when their defining sera were used on completely unrelated animals, and where recombinants might have been expected (Spooner et al 1979a; Anon, 1982). Another important observation is that of Teale et al (1985b), which appears to suggest that the sub-groups, rather than w6-broad, are the targets for primary MLC-generated alloimmune CTL. This is very strong evidence that w6-broad does not represent a single BoLA class I allele but rather a determinant common to several. Thus the evidence of CTL appears to contradict the interpretation of the w6 sub-groups as products of a second locus, which the serology suggests.

The simplest resolution of these two contradictory sets of evidence would be if the non-w6 occurrence of the subgroups was due to cross-reactivity with other BoLA products. It is true that the clustering of the defining sera is not very close in any population. Perhaps the first step in an attempt to answer the question of the control of these sub-groups would be to attempt further

absorptions of the defining sera, together with further immunisations to raise new sera, and the latter approach is now being started.

Another BoLA-A determinant which showed good evidence of sub-groups is w8. It seems clear that both EDA080 and KMA022 are detecting a fraction of the w8 animals, although in all populations these sera do react outside w8. This might be taken as evidence that the specificities which these sera detect are under the control of a second locus, although again, very high linkage disequilibrium would have to be postulated in two widely separated groups. Since these sub-groups were not recognised hitherto, no attempt has been made to remove non-w8 activity from the sera, or to absorb them to identify two mutually exclusive sub-groups. Until this has been done, further discussion is not useful. It is not known if w8-broad (as defined by EDA013 and EDA063) is a target for bovine CTL, and this is also be an important area for investigation.

w10 is another cluster which showed evidence of subgroups. The sub-group M4 is defined by two reagents, a xenogeneic MoAb (MA 4.1) and an alloantiserum (KKA004). Their reaction patterns are almost identical, and they do react without w10 at a significant frequency. This might be taken as evidence that M4 is under second locus control. However, 10 N'damas from The Gambia were tested during this study and four of them were w10. Of these, all four were both KMA003 and M4 positive. Once again a

remarkable linkage disequilibrium must be postulated for M4 to be a second locus product. The picture in the European cattle is rather different. MA 4.1 was not tested in Europe during this study, but KKA004 has since been tested in Edinburgh against 332 Bos taurus animals. KKA004 reacted with five animals, only one of which was w10. There were 62 w10 animals in this group (Spooner, personal communication). Forty nine Friesian/Sahiwal crosses were tested in this study. They were of unknown relatedness but it may be significant that none were MA 4.1 or KMA003 positive, but 14 were w10. It is of course possible that all of the w10's came from the Friesian element in these animals, but this observation would suggest that further testing of pure Sahiwals might be informative.

If the M4 specificity is under second locus control, then important questions are raised about its linkage with w10 in African cattle. N'damas are possibly no more closely related to Zebus and Borans than they are to European animals (Epstein, 1971). Such a close linkage between M4 and w10 in these animals and other African cattle would be strong evidence that there is a survival advantage in these determinants occurring together which is absent in European animals.

There is, however, an alternative explanation which does not require linkage disequilibrium of such unlikely tenacity. M4 may simply be common to more than one BoLA

product. This explanation requires that a "very private" determinant (in that it is present on only a fraction of w10 animals) occurs on two distinct molecules. There is no reason why this should not happen, and such complex patterns of cross-reactivity are known between HLA antigens (Dausset, 1981b). There are a number of ways in which such a situation might occur, the simplest being an intragenic recombination (Bodmer, 1984) between w10 and another specificity carrying the M4 determinant. If this were so, then either this event occurred once and the w10,M4 determinant was subsequently lost in European cattle, or it may have occurred independently in two widely separate African populations. Either explanation suggests that the M4 determinant might have significant survival value. This explanation does however seem more likely than that of two-locus control, and it is supported by preliminary evidence that KKA004 and an anti-w10-broad antibody precipitate the same molecules in a w10/M4 animal (Bensaid, personal communication).

Another important fact is that the KM3 determinant is absent in European cattle and present in almost all African w10 animals. It seems likely that this is a determinant on the w10 molecule, and its total absence in Europe may reflect a survival attribute associated with w10 in the African environment. It may also be a purely chance "loss" from the European w10.

w10 is serologically well defined in both Europe and Africa, but there appears to be a major difference between

w10 in the two populations. In Africa - in Boran, Zebu and West African N'dama - the vast majority of w10 animals are positive for both KMA003 and MA 4.1. In Europe, KM3 may not occur and M4 appears to be completely independent of w10.

Cytolytic T-cells have been valuable tools when considering the nature of these sub-groups. In cattle, both alloreactive (Teale et al, 1985a; 1985b) and autologous anti-Theileria (Morrison et al, 1985) T-cells have been reported. Both have shown restriction of cytotoxicity to the serologically defined BoLA specificities, and some clones have provided evidence of specificity for subgroups of these antigens. This is analogous with experience in the human field, where such CTL have tended to split existing specificities (Lopez de Castro et al, 1984).

To take the example of HLA-A2, Wallace et al (1985) showed allospecific T-cells which detected sub-types of A2, and those with specificity for broad A2. Even xenogeneic mouse-anti human CTL can distinguish these variants (Engelhard and Benjamin, 1983). However, these A2 variants have not so far been detected serologically, and this has prompted Wallace et al (1985) to suggest that there are epitopes detected by CTL but not alloantisera. There is no evidence for such epitopes in the bovine class I molecules, for while CTL have suggested splits of existing specificities, there has always been serological

evidence of such sub-groups. This may be a reflection of the advantages of the planned immunisations and absorptions which are possible in cattle but not humans.

Population analysis

Two groups of African cattle were tested in useful numbers, Zebus and Improved Borans. These groups are certainly closely related. The Boran is simply a Zebu which has been selected for beef production under modern ranching regimes for perhaps 30 generations. There is evidence that they have a lowered resistance to East Coast fever and drought and have a reduced walking ability by comparison with the original Zebu stock (Young and Dolan, personal communication).

The Zebu cattle came from an extremely wide range of areas and were very uniform in their BoLA profile. The Borans, in some respects, resembled European cattle more closely than the Zebus. It is important to refine the definition of BoLA in these groups in order to allow the identification of specificities which may have a changed frequency in the Borans, possibly the result of selection for high productivity under reduced environmental stress. The alleles which have not changed in frequency may be more important than those which have, in that they might be associated with tolerance to some diseases to which European animals are extremely vulnerable. Two obvious candidates for this are w12 and w16. These specificities are extremely rare in both African groups. The fact that

their frequencies are unchanged by selection could be chance, or it might indicate that they are unsuited to the African environment. Most authors (Epstein, 1971; Mason and Maule, 1960) admit the possibility that the Boran contains some Bos taurus blood. If this is so, then the w12 and w16 which might have been introduced has been all but lost.

The major differences between the African and European cattle in the frequencies of the BoLA-A alleles are the relatively low frequency of w3, w12, w16 and the w6 sub-groups and the high frequency of KM18 in the African cattle. These clusters would therefore seem to be the most important to include in further studies of these cattle, with the aim of determining the characteristics of the animals which carry them.

The future

This study has succeeded in reducing the frequency of the null allele in African cattle, particularly Borans, to one which is comparable to that of European cattle. It is now possible to embark on studies of the immune system of these cattle without the need to confine studies to atypical examples with identifiable BoLA alleles.

However, there is much remaining to be done. There may be more class I loci to be described, and there are certainly more BoLA-A locus alleles. It is important to extend the knowledge of BoLA further. Without the most complete understanding of this fundamental system,

important associations which may exist between BoLA and disease resistance are likely to be missed.

This study has highlighted a number of areas where absorption analysis of the existing sera would be extremely valuable, particularly by absorbing with cells from cattle outside the population in which the sera were made. Simply using cattle from differing herds is clearly important, but the use of different races has been extremely revealing. There are a number of obvious areas where more sera could usefully be raised, particularly in an attempt to answer the question of sub-groups.

Calves obtained by embryo transplant are likely to make an invaluable contribution to the understanding of the genetic control of BoLA, and allow the identification of further loci. This approach is the only one which would allow recombinants to be identified with certainty.

Monoclonal Antibodies

Monoclonal antibodies seem likely to have an important future in this work. This study describes the only such reagent presently available which detects a "private" BoLA product.

The application of monoclonal technology to HLA-typing is reviewed by Bodmer and Bodmer (1984b). It is clear that MoAbs are becoming of great value in tissue typing, but are still far from displacing alloantisera. At the ninth HLA workshop (Albert et al, 1984), MoAbs were

important in antigen definition for the first time. They have generally exhibited the same patterns of cross-reactivity as have alloantisera; they have suggested splits of existing specificities; and a MoAb-defined specificity has appeared (HLA-Aw69) (Fauchet et al, 1984). However, it is also clear that there are potentially serious technical problems in the use of these tools. Bodmer et al (1984) cite two major hazards; carry-over and loss of reactivity.

This study, and other preliminary investigations of the application of MoAbs to BoLA-typing (Morrison and Teale, personal communication) suggest that these problems can readily be overcome. The crucial stage in MoAb generation is the screening. Bodmer and Bodmer (1984b) suggest that the cytotoxicity test is not ideal for this purpose, as many useful antibodies do not fix complement. This however, seems an unrealistic approach. Culture supernatants can readily be screened in the cytotoxicity test within a few days of the initial fusion, and this allows the rejection of the vast majority as monomorphic or non cytotoxic. The major problems associated with the growth of MoAbs only become evident after this stage, and it seems more satisfactory to expend resources only on potentially ideal antibodies. Early screening may also reject MoAbs which give poor reproducibility in the cytotoxicity test. In this way a panel of high quality reagents may rapidly be acquired.

The problems associated with carry-over of MoAbs into adjacent wells do not seem insurmountable. The use of tissue culture supernatants rather than ascites, care in the dispensing of cells and complement and the monitoring of carry-over by intervening control wells, have proved entirely successful in this study.

A typing panel based on MoAbs, while considerably more difficult than a conventional panel to generate, will have the great advantage of immortality. Most alloantisera are available in quantities of only a few ml, and this means that any laboratory working on BoLA must be continually maintaining its stocks of sera with the constant problem of not having standard reagents. Furthermore, it does not seem unreasonable to expect increased reproducibility from a panel of MoAbs by comparison with antisera, provided they are screened for a potential loss of reactivity in storage or transport. This is because, by using them at a titre well above their end-point, variations due to the complement source may be all but eliminated.

It should be remembered, however, that the epitopes against which a mouse generates antibodies may not be the same as those seen by cattle, and for this reason alloantisera must be maintained in parallel with MoAbs. Human monoclonal human antibodies are now being investigated, but present some technical difficulties (Cole et al, 1985). A bovine fusion partner would be highly desirable for preparing BoLA reagents. This

approach would be expected to be even more valuable than the use of murine antibodies, as it will reduce the relative number of monomorphic determinants which the immunised animal may recognise as non self.

Micro-computers

This study was considerably aided by the use of a micro-computer. In the past, the analysis of tissue typing data has been performed on a "one off" basis, where a mass of results has been given to computer technicians who then analysed it on a main-frame computer. This approach has many disadvantages, the greatest of which is that it is extremely difficult to use the computer in a "conversational" way, whereby a series of questions can be asked of the data, each question depending upon the answer to the last.

The power and relative cheapness of the modern micro-computer now means that the investigator himself can have unrestricted access to the data and, given good programmes, can have instantly available the results of thousands of tissue typings which can be examined and analysed in many different ways.

The programmes developed during this study proved to be of immense value in answering spontaneous questions of the sort, "how many w3's are EDA097 positive in male Borans". The software is constantly being refined and it seems certain that the micro-computer in the laboratory

will greatly aid the developement of tissue typing. The fact that the investigator who understands the problems, rather than the professional statistician and software engineer, can now be responsible for data analysis, can only be beneficial.

The modern micro-computer should not therefore be seen as an improvement on the main-frame, but rather as a new and valuable tool for the laboratory. Its uses are qualitatively different from those of the main-frame and its potential use in tissue typing is illustrated by reports of micro-computer controlled assay systems which appear to be accurate and reliable (Bruning et al, 1982). Beyond this however, its potential for database management and simple analysis is considerable.

A role for BoLA-typing ?

The results of this study support the theory that MHC alleles are exposed to natural selection as a result of their role in the immune system. It is an increasingly accepted view that the so-called immune response (Ir) genes are actually the MHC class I and II genes themselves (Klein et al, 1983). Cattle in Africa are certainly under powerful selection from a wide range of diseases. The African cattle in this study will have been exposed for many generations to tick and tsetse borne haematoprotozoa which do not occur in Europe.

Thus, further study of BoLA in African (and European)

cattle seems to be important. Sophisticated regimes of prophylaxis and treatment are not always suited to the management systems of Africa. Attempts to "improve" the stock by blindly introducing exotic animals seem futile. The exploitation of innate resistance, which would be greatly facilitated by marker genes, offers the only sensible alternative.

CONCLUSIONS

Previously undetected sub-groups of two internationally recognised BoLA specificities have been demonstrated. These are ED80 and KM22, which are splits of w8, and KM3 and M4 which are splits of w10. The behaviour of the sub-groups of w6 was examined, and their frequency was found to be considerably lower in African than in European cattle, while the frequency of the w6 determinant was similar in all groups.

Five new BoLA-A locus alleles were detected. The frequency of two of these (KM8 and KM12) was high in both African populations and extremely low in European cattle. Large differences were found between different populations in the antigen frequency of all specificities except w6 and w8. The apparent specificity of the highly refined European BoLA-typing sera was dramatically reduced when they were used to type African cattle.

No good evidence was found for the existence of further bovine class I loci, and this may suggest that such loci, if they exist, are monomorphic.

A mouse monoclonal antibody (MA 4.1) is described, which has specificity for a split of w10 and which has a close correlation with an alloantiserum (KKA004). The future role of monoclonal antibodies in BoLA typing seems great.

A new means of cryopreservation of lymphocytes under field conditions was developed and it played a major role in providing material for this work.

A series of computer programmes was written which allowed a micro-computer to store and analyse the data which were acquired. Algorithms were created which used a new approach to the clustering of antisera, allowing improved identification of sera with overlapping activity.

Comparison with the HLA system suggests that the BoLA antigens are similar in many respects to those of the human. They show similar splits and are readily detected by cytolytic T cells. This study has succeeded in improving the definition of BoLA in East African cattle to the point where the frequency of undetected alleles is no greater than that in most European breeds. It is now possible to conduct population studies and specific experiments aimed at identifying MHC products which are associated with disease susceptibility or resistance.

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Appendix 1: Suppliers

AST Research, Irvin, California, USA.

Bayer, Leverkusen,Germany.

Becton Dickinson, Rutherford, New Jersey, USA.

Cedar Lane, Hornby, Ontario, Canada.

Costar, Cambridge, Mass, USA.

Fisher, New Jersey, USA.

Gelman, Ann Arbor, Michigan, USA.

Gibco, Uxbridge, Middlesex, UK.

Hamilton, Bonaduz, Switzerland.

Kobian Ltd, Nairobi, Kenya.

Micro Pro International, San Rafael, California, USA.

Millex, Molsheim, France.

Nunc, Denmark.

Pharmacia, Uppsala, Sweden.

Pharmeacutical Manufacturing Company, Nairobi, Kenya.

Prospero Software, 190 Castelnau, London, UK.

Sigma, St Louis, USA.

Sterilin, Teddington, Middlesex, UK.

Venoject, 3030 Leuven, Belgium.

Appendix 2: Pascal source code of BoLA programmes

There follows a listing of the Pascal source code used to create the BoLA analysis programmes. This code was written for the Prospero Pascal compiler (version 1.1) (Prospero Software) and can only be compiled directly by this compiler running on an IBM-PC or compatible micro-computer. However, Pascal is a particularly machine independent language and other compilers for other machines could be used if the code was slightly modified. The parts most likely to give problems are the file handling routines, routines designed to modify screen and printer i/o and the Prospero routines such as date. The bulk of the code will certainly be usable on other machines without modification.

The screen and keyboard control routines require that the machine be started with a "CONFIG.SYS" file in the root directory, containing the line "DEVICE=ANSI.SYS".

The Pascal Source Code

The source code for the BoLA analysis progammes is shown below. Many of the lines of the original programmes are too long to fit on the page and have therefore been broken. In general Pascal is very tolerant of line breaks, however it has sometimes been necessary to break a variable name in the middle and frequently it has been necessary to break a literal string (ie text within quotes). If used exactly as it is below the source code could not be successfully compiled because these two conditions would create errors. It has also been necessary to considerably alter the layout so the classical structuring of Pascal programmes into nested segments has been largely destroyed.

THE MENU

```
program BOLA_MENU (input,output);
var x:integer;
PROCEDURE Clearscreen;EXTERNAL;

begin
CLEARSCREEN;
writeln;writeln;
write('                    ',
chr(1BH),'[7m' ,'BoLA  [by SJK 2-10-84]');
write(chr(1BH),'[0m',chr(1BH),'[1m');
for x:=1 to 6 do writeln;
writeln('                Similarity index.....1');
writeln('                Print out data.......2');
writeln('                Update/review data ..3');
writeln('                Reorder sera in a file....4');
writeln('                Enter data.............5');
writeln('                Select data............6');
writeln('                Compare serum frequencies.7');
writeln('                Assign clusters........8');
writeln('                Join BoLA files........9');
writeln('                Convert IBM to PRIME.....10');
writeln('                Convert PRIME to IBM.....11');
writeln;
WRITELN('                Quit....................0');
write(chr(1BH),'[4m');
writeln;

repeat
write('choice: ');readln(x);
until ( (x>=0) and (x<=11) );
```



```
write(chr(1BH),'[0m');
case x of
1 :chain('sim');     2 :chain('printout');
3 :chain('update'); 4 :chain('switch');
5 :chain('entry');  6 :chain('select');
7 :chain('chi-sq'); 8 :chain('cluster');
9 :chain('Pas-pas');10:chain('pas-for');
11:chain('for-pas');0 :WRITELN;
end;
end.
```

SIMILARITY INDEX

```
Program Turbo_Similarity
(input,output,datafile,serafile,printer);

{A HIGH SPEED VERSION OF SIMILARITY WHICH USES A RAM DISK
TO HOLD THE TOTALS FOR THE 2X2 TABLE ALLOWING FOR MANY
SERA TO BE COMPARED WITH ALL OTHERS IN A SINGLE PASS
THROUGH THE DATA.  THE NUMBER OF SERA THAT CAN BE
COMPARED IS GOVERNED BY THE SIZE OF THE RAM DISK - EACH
SERUM WILL  OCUPY 480 BYTES ON EACH OF 4 FILES.
THEREFORE NUMBER OF SERA = RAM DISK CAPACITY  DIVIDED BY
1920}

CONST   Allsera      =240;        {max number of sera}
        Serum_length=  6;         {length of serum names}
        Name_length = 40;         {length of animals name}
        Page_length = 35;         {number of lines printed
                                   per page}

TYPE    One_to_ten  =1..10;
        One_to_eight=1.. 8;
        One_byte    =0..253;  {integer range for 1 byte
                               storage}
        Two_byte    =0..65533;{integer range for 2 byte
                               storage}
        Maxno       =1..Allsera;
        Set_of_fields=(batch,date,sex,breed,location,
                       sublocation,CBTS,spare);
        Set_of_scores=SET OF CHAR;
        Panel=Array[maxno]
              of packed array[1..serum_length] of char;
        worktype=     Array[maxno] of two_byte;

        Rec_of_results=RECORD
               Name:Packed array[1..Name_length] OF CHAR;
            Details:Array[set_of_fields] OF Packed
                    array[One_to_eight] of char;
             Serums:Array[Maxno] OF Char;
                    END;

        BOLAfile=FILE OF Rec_of_results;
        Workfile=FILE of worktype;
```

```
VAR       Results:          Rec_of_results;
          Test_serum:       packed array
                            [1..serum_length] of char;
          Serum_panel:      panel;
          Seen:             Two_byte;
          One_pos,
          Two_pos,
          Both_pos,
          No_pos:           Workfile;
              {work files holding the counts}
          Array_filler:     Worktype;
          drive:            String[2];
         {to hold the SUPERDRIVE drive designator}
          min,highlight,
          X,Count:                  One_byte;
          Within,
          Found,Opened:             Boolean;
          Eachserum,
          Panel_size:               Maxno;
          Posscores:                Set_of_scores;
          Test:                     Array[1..2] of maxno;
          Message,
          printerfilname,
          Serafname,
          Datafname:                STRING[14];
          Datafile:                 BOLAfile;
          printer,
          Serafile:                 TEXT;
          bottom1,bottom2,
          bottom,top,r,y,simx:      LONGREAL;
FUNCTION YESNO (Default:CHAR; Message:STRING):
                                BOOLEAN;EXTERNAL;
PROCEDURE Clearscreen;                EXTERNAL;
PROCEDURE Bleep;                      EXTERNAL;
PROCEDURE Bright;                     EXTERNAL;
PROCEDURE Normal;                     EXTERNAL;
FUNCTION Caseup(ONECHAR:CHAR):CHAR;EXTERNAL;
PROCEDURE Wipe(X:integer);            EXTERNAL;
FUNCTION Namecheck(filename:string):
                               Boolean;EXTERNAL;
BEGIN {MAIN PROGRAMME}
clearscreen;
FOR X:=1 TO 8 Do writeln;
writeln('          ***********************  ');
writeln('          Turbo Similarity Index   ');
writeln('          ***********************  ');
writeln;writeln;writeln;writeln;writeln;
writeln('sjk 28/8/84');
For Eachserum:=1 to 240 do
begin
For X:=0 TO 252 do;
end;
clearscreen;
FOR Count:=1 to allsera do
begin
```



```
For X:=1 to Serum_length do Serum_panel[Count] [X]:=' ';
end;
For X:=1 To Serum_length do Test_serum[X]:=' ';
{open the work files on SUPERDRIVE}
write('enter RAM drive designation (eg B:): ');
readln(drive);WRITELN;
assign(One_pos, (concat(drive,'work1')) );
assign(Two_pos, (concat(drive,'work2')) );
assign(Both_pos,(concat(drive,'work3')) );
assign(No_pos,  (concat(drive,'work4')) );
update(one_pos);
update(two_pos);
update(both_pos);
update(No_pos);
{now open the file with the data}
REPEAT
  REPEAT
    write('File with BoLA data');bright;
    write(' [           ].DTA ');
    write(chr(1BH),'[16D');
    readln(datafname);writeln;normal;
  UNTIL(namecheck(datafname));
  datafname:=concat(datafname,'.DTA');
  IF NOT Fstat(datafname) THEN
  writeln ('There is no file ',datafname);
Until (Fstat(datafname));
Assign(datafile,datafname); writeln;
{open the file holding the serum panel}
REPEAT
  REPEAT
    write('File with serum batch');bright;
    write(' [           ].BCH ');
    write(chr(1BH),'[16D');
    readln(serafname);writeln;normal;
  UNTIL(namecheck(serafname));
  serafname:=concat(serafname,'.BCH');
  IF Not Fstat(serafname) Then
  writeln ('There is no file ',serafname);
UNTIL (Fstat(serafname));
Assign(serafile,serafname); reset(serafile); writeln;
REPEAT
  writeln;Opened:=TRUE;
  Write('Output file (printer = "PRN"): ');
  Readln(Printerfilname);
  IF Printerfilname='prn' then Printerfilname:='PRN';
  {simplify the next if !}
  IF (Printerfilname<>'PRN') AND Fstat(Printerfilname)
  THEN
     BEGIN
        IF Yesno('X','This file exists OK to erase it')
        Then   Opened:=TRUE
        ELSE   Opened:=FALSE;
     END;
UNTIL (Opened);
assign(printer,printerfilname);rewrite(printer);
```

```
IF (Yesno('x','count 4 as -ve') )Then
     Posscores:=['8','6']
ELSE Posscores:=['8','6','4'];
REPEAT
Write('minimum similarity index (%) for printing    ?:');
readln(min);
UNTIL ( (min>=0) AND (min<=100) );
REPEAT
write('minimum similarity index (%) for highlightng ?:');
readln(highlight);
UNTIL ( (highlight>=min) AND (highlight<=100) );
{fill the array serum_panel with the serum names in this
file}
Panel_size:=0;
WHILE (Panel_size<=Allsera) AND (NOT (eof(Sera_file))) DO
  BEGIN
        Panel_size:=Panel_size+1;
        Count:=1;
        While NOT eoln(Sera_file) and (Count <=6) DO
                BEGIN
                 READ( Sera_file,
                       Serum_panel [Panel_size] [Count] );
                 Count:=Count+1;
                End;
        READLN(Sera_file);
END;    {while panel size}
{the serum names are in the array Serum_panel}
{and the variable panel_size is equal to the number of
sera in the panel}
FOR Count:=1 TO serum_length DO Test_serum[Count]:=' ';
{now read the serum_names  to be compared}
REPEAT
  FOR X:=1 TO 2 DO
  BEGIN
    Found:=FALSE;
     REPEAT
       IF x=1 then
         write('enter the name of first serum:   ');
       IF x=2 then
         write('enter the name of last  serum:   ');
       Count:=1;
       While not(eoln(input))
       and (Count<=serum_length) DO
       BEGIN
       read (Test_serum[count]);
       Test_serum[Count]:= caseup( test_serum[Count] );
       Count:=Count+1;
       END;
     readln;
{find the position of this serum in the panel}
     Eachserum:=0;
       Repeat
       Eachserum:=Eachserum+1;
       Until  (serum_panel [Eachserum]=Testserum)
              OR (Eachserum>=allsera) ;
```



```
       IF (serum_panel [Eachserum]=Test_serum)
       THEN Found:=TRUE
       ELSE writeln(Test_serum,' not found in ',serafname);
       UNTIL (Found) ;
     Test[x]:=Eachserum;
     END;
IF NOT (Test[1]<=Test[2]) THEN
writeln ('Do not be silly - ',
serum_panel[Test[2] ],' is before ',
serum_panel[Test[1] ],' in the panel');
UNTIL (Test[1]<=Test[2]);
for x:=1 to allsera do array_filler[x]:=0;
{set the files that will hold the totals of the 2x2 table}
    For Eachserum:=test[1] to test[2] do
    begin
    write(one_pos,Array_filler);
    write(two_pos,Array_filler);
    write(both_pos,Array_filler);
    write(No_pos,Array_filler);
    writeln((Eachserum-Test[1]+1):1,
             ' records written to 4 files');
    end;
   clearscreen;
   reset(datafile);
   write(chr(1BH),'[21;10H','sera being compared');
   write(chr(1BH),'[7m');
   write(chr(1BH),  '[22;10H',Serum_panel[TEST[1]],' - ',
        Serum_panel[TEST[2]]);
   write(chr(1BH),'[0m');
     seen:=0;
     write(chr(1BH),'[21;40H','animals read');
     write(chr(1BH),'[7m');  {reverse video}
          {****  READ THE DATA FILE ****}
While not eof(datafile) do
BEGIN
 seek(One_pos,0);
 seek(Two_pos,0);
 seek(Both_pos,0);
 seek(No_pos,0);
  IF NOT (DATAFILE^.NAME[1]='£') THEN
  BEGIN  {** IGNORE ANIMALS WITH £ AS FIRST CHARACTER ***}
     seen:=seen+1;
     IF (seen=65533) then
     begin
       For X:=1 to 20 do write(printer,chr(07H));
       {MAKE A LOT OF NOISE}
       For x:=1 to 10 do Writeln(printer);
       For x:=1 to 10 do Writeln(printer,
'TOO MANY ANIMALS IN FILE MAX:65535 RESULTS MEANINGLESS');
       For x:=1 to 10 do Writeln(printer);
     end;
      write(chr(1BH),'[22;40H',seen:1);
    For Eachserum:=test[1] to test[2] do
    begin
      IF NOT (DATAFILE^.serums[Eachserum]='.') Then
```

```
      BEGIN{E}
         For Count:=1 to panel_size do
         begin{D}
           IF NOT (DATAFILE^.serums[Count]='.') THEN
           BEGIN{B}
              IF DATAFILE^.serums[Eachserum] IN Posscores
              Then
               Begin{A}
                 IF DATAFILE^.serums[Count] IN Posscores
               THEN   both_pos^[count]:=both_pos^[count]+1
               ELSE    One_pos^[Count]:=One_pos^[Count]+1;
               END{A}
               ELSE
               BEGIN{C}
                 IF DATAFILE^.serums[Count] IN Posscores
                 Then Two_pos^[Count]:=Two_pos^[Count]+1
                 ELSE No_pos^[Count]:=No_pos^[Count]+1;
               END;{C}
            END;{B}
         END;{D}
      END;{E}
      put(One_pos);
      put(two_pos);
      put(both_pos);
      put(no_pos);
     END;{For eachserum}
    END;{if not £      }
   GET(DATAFILE);
END;{WHILE NOT EOF}
write(chr(1BH),'[0m');{reverse video off}
For eachserum:=test[1] to test[2] do
begin
Writeln;
Write('S1: ',serumPanel[eachserum],'     S2: ');
  seek(One_pos, eachserum-test[1]);
  seek(two_pos, eachserum-test[1]);
  seek(both_pos,eachserum-test[1]);
  seek(no_pos,  eachserum-test[1]);
  x:=0;
  write(printer,chr(1BH),'D',CHR(ORD(90)),CHR(ORD(0)) );
  {SET TAB}
  writeln(printer,chr(0CH));
  {formfeed}
  write(printer,chr(1BH),'!',chr(ord(8)) );
  {set print style 8}
  write(printer,'from ',datafname);
  IF ('4'IN Posscores)THEN
  Writeln(printer,'                4 is considered +ve')
                  ELSE
  writeln(printer,'                4 is considered -ve');
  writeln(printer,'minimum simx printed: ',min:1,
  '%   minimum highlighted: ',
  highlight:1,'%');
  writeln(printer);
  writeln(printer,  '          similarity¦correlation');
```



```
  writeln(printer,
  's1    ¦  s2  ¦     s1 only   ¦ s2 only  ¦s1 & s2 ',
  ' ¦ neither¦total¦     index   ¦coefficient ¦');
  writeln(printer);
  {***************** CALCULATE *************************}
  {* SIMILARITY INDEX AND CORRELATION  FOR EACH  SERUM **}
  {*****************************************************}
  FOR Count:=1 to panel_size do    {LOOP FOR EACH SERUM}
  begin
    write(Count:3);write(chr(1BH),'[3D');
          {***CALCULATE SIMILARITY INDEX***}
    y:=( one_pos^[count] + two_pos^[count] +
                                 (2*both_pos^[count]) );
    IF NOT (y<=0) AND (Count<>Eachserum) THEN
    {avoid error Z and comparing a serum with itself}
    BEGIN
      simx:=(200*Both_pos^[count])  / y;
                {*** CALCULATE r ***}
      top:=    (both_pos^[count] * no_pos^[count]) -
                        (one_pos^[count]*two_pos^[count]);
      bottom1:=(both_pos^[count]+one_pos^[count])*
      (both_pos^[count]+two_pos^[count]);
      bottom2:=(no_pos^[count]+two_pos^[count])*
      ( one_pos^[count]+ no_pos^[count]);
      bottom:=bottom1*bottom2;
    {THE CALCULATION OF BOTTOM IS BROKEN UP TO AVOID
     OVERFLOWING INTEGER ARITHMETIC}
      If bottom>0 then         {AVOID SQR ROOT OF 0 ERROR}
      begin
        bottom:=sqrt(bottom);
        r:=top/bottom;
      end
      Else r:=0;
{* HOW MUCH IS ONE SERUM'S REACTIONS WITHIN ANOTHERS ?*}
      Within:= (both_pos^[count]>(10*one_pos^[count]) ) OR
                (both_pos^[count]>(10*two_Pos^[count]) );
      If Within then within:=(   (one_pos^[count]>5) OR
                                 (two_pos^[count]>5)   );
       Message:='-  within ?';
      If Within and (both_pos^[count]>10) Then
      BEGIN
        If (  (one_pos^[count]=0) OR (two_pos^[count]=0) )
                        Then Message:='** WITHIN **'
        ELSE                 Message:='-  WITHIN  ?';
      END;
      IF (simx>=min) OR Within then
      {***if simx > than min for printing***}
      begin      {***or evidence of containment***}
        IF (simx>=highlight)then
        write(printer,chr(1BH),'!',chr(ord(24)) );
        {if greater than min for highlighting then set
         printer to double strike}
         {*** WRITE OUT THE RESULTS TO THE PRINTER ***}
        Write(printer,Serum_Panel[Each_serum],
          ' ',serum_panel[Count],One_pos^[count],
```

```
        two_pos^[count],both_pos^[count],no_pos^[Count],
        ',(one_pos^[Count]+two_pos^[count]+
          both_pos^[count]+no_pos^[count]):4,
              ',simx:10:1,r:10:3 );
       IF Within THEN writeln(printer,'    ',message)
          {if evidence of containment}
       ELSE writeln(printer);
       write(printer,chr(1BH),'!',chr(ord(8)) );
       {reset printer to single strike}
       X:=X+1;
     end;
  END;
  IF (X=page_length) Then
  begin
    writeln(printer,chr(0CH));  {formfeed}
    x:=0;
  end;
end;{for Count    }
end;{for eachserum}
writeln(printer);writeln(printer);
writeln(printer);writeln(printer);
writeln(Printer,'                                KEY');
writeln(Printer,
'-------------------------------------------------------');
writeln(printer,
'within  ?....10x one serum alone',
' < both together and one serum alone >5');
writeln(Printer,
'WITHIN  ?....and more than 10 animals',
' react with both together');
writeln(Printer,
'** WITHIN **....and one serum never reacts alone');
writeln(Printer,
'-------------------------------------------------------');
writeln(printer);writeln(printer);
writeln(printer,'Total number of animals in ',
datafname,' is ',seen:1);
writeln;writeln('done');
writeln(printer,chr(0CH));                {formfeed}
write(printer,chr(1BH),'!',chr(ord(1)) ); {print style 1}
    erase(One_pos);
    erase(two_pos);
    erase(both_pos);
    erase(no_pos);
CHAIN('BOLA');
END.
```


Printout programme

```
PROGRAM Printout
(input,output,sourcefile,serafile,sequencefile,printer);

CONST
Max_per_page=44;    {lines of printout per page}
Printable=  187;    {number of sera whose reactions can be
                     shown}
Name_length= 40;    {number of characters in the NAME
                     field}
Field_length= 8;    {number of characters in the ID fields}
Serum_length= 6;    {number of characters in the serum
                     names}
Allsera=    240;    {max number of sera in the sourcefile}
Partname=    15;    {the number of characters in the name
                     to be printed}
Tab=         46;    {tab position at which printing of
                     scores begins}
TYPE
One_to_eight=1..8;
printcodes=0..63;
setofcodes=SET OF printcodes;
Maxno       =1..Allsera;
Cando       =1..Printable;
Set_of_fields=
(batch,date,sex,breed,location,sublocation,CBTS,spare);
PA=   PACKED ARRAY[1..Name_length] OF CHAR;
Rec_of_results=RECORD
Name:PA;
Details:Array[Set_of_fields] OF
     PACKED ARRAY [1..Field_length] OF CHAR;
     Serums:ARRAY[Maxno] OF CHAR;
        END;
Order_Array =Array[Cando] OF Maxno;
Heading_array=Array[Cando]OF CHAR;
Panel=Array[Maxno] OF
PACKED ARRAY[1..Serum_length] OF CHAR;
PS_array=PACKED ARRAY[1..Serum_length] OF CHAR;
BOLAfile=FILE OF Rec_of_results;
VAR
Results:         Rec_of_results;
unique_codes:    setofcodes;
Onechar:         char;
Order:           Order_array;
Title,Heads:     Heading_array;
Serum_panel:     Panel;
To_print:        Cando;
Opened,Found:    BOOLEAN;
Print_serum:     PS_array;
Eachserum:       Maxno;
pos1,pos2,         {first and last characters in the name}
X,line,
yy,dd,           {to hold the date}
Page,Count:      Integer;
```

```
Sourcefile:      BOLAfile;
Serafile,
Sequencefile,
Printer:         TEXT;
Printerfilname,
ms,Sourcename,
Serafname,
Seqfname:        STRING;
FUNCTION YESNO (Default:CHAR; Message:STRING):
BOOLEAN;EXTERNAL;
PROCEDURE Clearscreen;  EXTERNAL;
PROCEDURE Bleep;        EXTERNAL;
FUNCTION Caseup (letter: CHAR):CHAR;EXTERNAL;
PROCEDURE pasdate(VAR year,day:INTEGER;VAR month:STRING);
EXTERNAL;
FUNCTION len (var pos1,pos2:Integer;
              TestString: PA):Boolean;
{returns positions of first (pos1) and last (pos2)
non blank characters in an array}
Var X         :  integer;
    not_blank:  boolean;
begin
len:=true;
{FIND REAL LENGTH OF STRING1}
{first find position of first non blank}
not_blank:=false;x:=1;
While (X<=40) and not (not_blank) do
begin
  If (Teststring[x]<>' ')
  Then not_blank:=True Else x:=x+1;
end;
IF (x=41) then len:=false
ELSE pos1:=x;
{FIND LAST NON BLANK}
x:=40;not_blank:=false;
while(x>=1) and not (not_blank) do
begin
  if (Teststring[x]<>' ')
  then not_blank:= TRUE Else  x:=x-1;
end;
pos2:=x;
end;{len}
BEGIN
Clearscreen;
FOR X:=1 TO 8 DO Writeln;
writeln('                          **********************');
writeln('                            BoLA DATA PRINT-OUT ');
writeln('                          **********************');
writeln;writeln;writeln;
writeln('complaints and comments to sjk');
FOR x:=1 TO 80000 DO;  {a short pause for thought}
unique_codes:=
          [0,1,4,8,16,17,20,24,32,33,36,40,48,49,52,56];
Clearscreen;
REPEAT
```



```
writeln;
write('File to be printed: ');
readln(sourcename);
IF NOT Fstat(sourcename) THEN
Writeln('There is no file ',sourcename);
UNTIL (Fstat(sourcename));
Assign(Sourcefile,Sourcename);
reset (sourcefile);
REPEAT
writeln;
write('file with serum names(the batch): ');
readln(serafname);
IF NOT Fstat(serafname) THEN
Writeln('There is no file ',serafname);
UNTIL (Fstat(serafname));
Assign(serafile,serafname);
reset(serafile);
REPEAT
writeln;
write('file with print sequence: ');
readln(seqfname);
IF NOT Fstat(seqfname) THEN
Writeln('There is no file ',seqfname);
UNTIL (Fstat(seqfname));
Assign(sequencefile,seqfname);
reset(sequencefile);
REPEAT
  writeln;Opened:=TRUE;
  Write('Output file (printer = "PRN") ');
  Readln(Printerfilname);
  IF Printerfilname='prn' then Printerfilname:='PRN';
  {simplify the next if !}
  IF (Printerfilname<>'PRN') AND Fstat(Printerfilname)
  THEN
     BEGIN
        IF Yesno('X','This file exists OK to erase it')
         Then Opened:=TRUE
         ELSE Opened:=FALSE;
     END;
UNTIL (Opened);
Assign(printer,'PRN');Rewrite(printer);
  {Printer is assigned to the printer}
  {at this stage no matter what o/p  }
  {file is chosen - it is reassigned }
  {to printerfilename before data is written}
{fill the title array}
for x:=1 to printable do title[x]:=' ';
writeln(printer); x:=1;
Writeln('Enter the title for the printout (up to ',
         printable:1,' characters)');
write('Title: ');
while not eoln(input) and (x<=printable) do
begin
  read(title[x]);
  x:=x+1;
```

```
end;
readln;
FOR To_print:=1 TO Printable DO
         Heads[To_print]:=' ';
        (set the headings to blanks)
FOR Count:=1 TO allsera DO
BEGIN
FOR X:=1 TO serumlength DO serum_panel[Count][x]:=' ';
END;
                                    (The 'DIVIDE' symbols)
FOR Count:=1 TO Serum_length DO Serum_Panel [240] [Count]
:=':';
FOR Count:=1 TO Serum_length DO Serum_Panel [239] [Count]
:=' ';
Eachserum:=1;
WHILE NOT eof(serafile) AND (Eachserum<=(Allsera-1)) DO
BEGIN
  Count:=1;
  WHILE NOT EOLN(SERAFILE) AND (COUNT<=SERUM_LENGTH)DO
        BEGIN
          Read(serafile,Serum_panel[Eachserum][Count]);
          Serum_panel[Eachserum][Count]:=
   caseup(Serum_panel[Eachserum][Count]);
          Count:=Count+1;
        END;
  Readln(serafile);
  Eachserum:=Eachserum+1;
END;
To_print:=1;
WHILE NOT eof(sequencefile) AND (To_print<=Printable) DO
BEGIN
  For Count:=1 TO Serum_length DO Print_serum[Count]:=' ';
  Count:=1;
  While not eoln(sequencefile)
  AND (Count<=Serum_length) DO
    BEGIN
      Read(sequencefile,Print_serum[Count]);
      Print_serum[Count]:=caseup(Print_serum[Count]);
      Count:=Count+1;
    END;
  READLN(SEQUENCEFILE);
  IF (Print_serum[1]='*') or (print_serum[1]=':') THEN
  (the divide symbols)
        BEGIN
        if print_serum[1]='*' then Order[To_print]:=239
        else order[to_print]:=240;
        Count:=To_print;
        FOR X:=2 TO Serum_length DO
                BEGIN
                  IF Printserum[X]<>' 'THEN
                  Heads[Count]:=Printserum[X];
                  Count:=Count+1;
                END;
        END
  ELSE BEGIN (the heading is a serum name)
```



```
        Count:=0; Found:=FALSE;
        WHILE (Count<=(Eachserum-1)) AND NOT (Found) DO
        BEGIN
           Count:=Count+1;
          If Print_serum = Serum_panel[Count] THEN
          (if the 2 names match)
                BEGIN
                 Order[To_print]:=Count;
                 (then this is the next heading)
                 Found:=TRUE;
                END;
        END;     (all serum names compared)
        IF NOT Found THEN BEGIN
                        Writeln(Printserum,' not found');
                        To_print:=To_print-1;
                        END;
         END;
         To_print:=To_print+1;
END;
To_print:=To_print-1;
(The sequence of printing has now been established in the
array ORDER and the headings are in the array HEADS)
Writeln
('  PRINT  STYLE  (for EPSON FX-80 & FX-100 printers)');
Writeln;
Writeln(to_print:1,
' sera and dividers plus ',
TAB:1,' columns for id details.');
Writeln('            TOTAL of ',
(To_print+TAB):1,' columns required.');
Writeln('                Code      ');
Writeln('          1 strike  2 strike ');
Writeln('Condensed      4      20     allows  233 columns.');
Writeln('Elite          1      17     allows  163 columns.');
Writeln('Pica           8      24     allows  136 columns.');
Writeln('Enlarged       32     48     allows  68  columns.');
Writeln;
Writeln('For a demonstration of each style enter 0.');
REPEAT
Write('choose the print code (0-63): ');
Readln(Count);
     BEGIN
        writeln(printer,chr(1BH),'D',chr(ord(20)),
                chr(ord(0)) );(sets the tab)
        For X:=0 TO 56 Do
        BEGIN
          IF(X IN Unique_codes)THEN
          BEGIN
          Writeln(printer);
          Writeln(Printer,chr(1BH),'!',chr(ord(X)),
          (sets the print mode)
        'Mode ',X:3,chr(09H),'sample text  SAMPLE TEXT');
          END;
        END;
     END;
```

```
UNTIL ((Count>=1) AND (Count<=63));
{*******************************************************}
{          begin the printing with the headings         }
{*******************************************************}
Assign(Printer,Printerfilname); Rewrite(Printer);
Writeln(printer,chr(1BH),'!',chr(ord(Count)),
     chr(1BH),'D',chr(ord(TAB)),chr(ord(0))  );
     {set the print mode and the tab}
writeln;writeln;
write('Wait');
pasdate(yy,dd,ms);
Page:=0;
REPEAT
Line:=0;
Writeln(printer,chr(0CH));                          {formfeed}
Writeln(printer);Writeln(printer);
Page:=Page+1;
Write(Printer,'Print out of file     ',sourcename,
'                                        Page',Page:4,
'             prepared  ',dd:1,'/',ms,'/',yy:1,chr(0DH));
Writeln(Printer,'_____________________________________');
Write(Printer,'Serum batch is        ',serafname,chr(0DH));
Writeln(Printer,'_____________________________________');
Write(Printer,'Using serum sequence ',seqfname,chr(0DH));
Writeln(Printer,'_____________________________________');
write(printer,chr(ord(137)) );  {TAB}
for count:=1 to printable do write (printer,title[count] );
writeln(printer);
write(printer,chr(ORD(137)) );  {Tab}
For Count:=1 TO To_print DO Write(Printer,Heads[Count]);
Writeln(printer);
FOR Count:=1 to Serum_length DO
        BEGIN
        If Count=serum_length then
write(printer,
'     NAME          DATE   BREED    LOCTN   SEX');
        write(printer,chr(ORD(137)) );  {Tab}
        FOR Eachserum:=1 TO To_print DO
Write(Printer,Serum_panel[order[Eachserum]] [Count]);
        writeln(printer);
        END;
write(printer,chr(ORD(137)) );  {Tab}
For Count:=1 To To_print DO Write(Printer,'-');
Writeln(printer);
{*******************************************************}
{now read the file and print it out                     }
{*******************************************************}
WHILE NOT eof(Sourcefile)AND (line<=Max_per_page) DO BEGIN
Read(Sourcefile,Results);
Results.Serums[240]:='!'; Results.Serums[239]:=' ';
IF NOT (Results.Name[1]='£') THEN
BEGIN
WITH Results do
   begin
   Found:=len(pos1,pos2,name);
```



```
  {find the first and last characters in the name}
   For x:=pos1 to (pos1+partname) do
   Write(Printer,Name[x]);
{write the first partname characters}
   If (pos2>(pos1+partname)) then
   write(printer,'>') else write(printer,'!');
{if are more than partname characters in the name then
show this with a ">"}
   write(printer,
   details[date],' '
 ,details[breed],' '
,details[location],' '
,details[sex][1],details[sex][2]);
   Write(printer,CHR(ORD(137))  );
   FOR Eachserum:=1 TO TO_PRINT DO
        Write(Printer,Serums[order[Eachserum]]);
   WRITELN(PRINTER);
   end;
END;
line:=line+1;
END;    {while not eof}
write('..')
UNTIL (eof(sourcefile));
Writeln(printer,chr(0CH));                          {formfeed}
write  (printer,chr(07H),chr(07H),chr(07H));
{ring the printers bell   }
Writeln(printer,chr(1BH),'@');
{re-initialise the printer}
write('DONE');
Chain('BoLA');
END.
```

```
PROGRAM NEWUPDATE (INPUT,OUTPUT,INFIL,OUTFIL,batchfile);

CONST   ALLSERA          =240;
        NAMELENGTH       =40;
        Serum_length     =6;
        Detail_length    =8;
        Selectable       =50;
{max number of sera which can be selected for}
 TYPE
Maxno            =1..Allsera;
Set_of_fields    =
(batch,date,sex,breed,location,sublocation,
                   CBTS,Spare);
PA               =Packed Array[1..name_length] of char;
PA8              =Packed Array[1..detail_length] of char;
Rec_of_results=RECORD
  Name:PA;
  Details:Array[set_of_fields]
          of packed array [1..detail_length] of char;
  Serums:Array[maxno] of char;
  END;
Srmname =          Packed Array[1..serum_length] OF CHAR;
Panel=            Array[maxno] OF Srmname;
{holds panel sera names}
BOLAfile         =File Of Rec_of_results;
ok_scores=       SET OF CHAR;
COMMON  Serum_Panel:     Panel;
         {this array is needed by procedure REVISE}
         {it holds the names of the panel sera     }
        Panel_size:      Maxno;
         {so is this it holds the number of sera in panel}
VAR
ID_field:         Array[setoffields]
                 of packed array[1..detail_length] of char;
seen,todo,
eachserum,moved,
Pos3,
Pos4,Count,X:    integer;
posn1,posn2:     array[1..selectable] of integer;
Choice:          char;
Field:           Set_of_fields;
Results:         Rec_of_results;
Continue,
GoForName,ok,
Found,serumonly,
Out,Opened:      BOOLEAN;
test:            array[1..selectable] of maxno;
Wanted:          Array[1..selectable] OF Boolean;
Select:          Packed Array[1..serum_length] OF char;
                 {names of selecting sera}
Pos_scores:      OK_scores;
S_name:          array[1..selectable] of PA;
S_field:         array[1..selectable] of PA8;
```



```
                 {as S_name for the id field}
batchfilname,
infilname,
Outfilname:      string[12];{the names of the files on disk}
Screen:          File Of Char;
                 {gives one chracter entry without <CR>}
Batchfil:        TEXT;
Infil  :         BOLAfile;  {the pascal non text files}
OK_choice:       SET OF 'A'..'Z';
FUNCTION YESNO (Default:CHAR; Message:STRING):BOOLEAN;
EXTERNAL;
FUNCTION Caseup (letter: CHAR):CHAR;EXTERNAL;
Function len (var pos1,pos2:Integer;
                 TestString: PA):Boolean;
{returns positions of first (pos1) and last (pos2)
non blank characters in an array}
Var X         :  integer;
    not_blank:  boolean;
begin
len:=true;
{FIND REAL LENGTH OF STRING1}
{first find position of first non blank}
not_blank:=false;x:=1;
While (X<=40) and not (not_blank) do
begin
 If (Teststring[x]<>' ') Then not_blank:=True Else x:=x+1;
end;
IF (x=41) then len:=false
ELSE pos1:=x;
{FIND LAST NON BLANK}
x:=40;not_blank:=false;
while(x>=1) and not (not_blank) do
begin
  if (Teststring[x]<>' ')
  then not_blank:= TRUE Else  x:=x-1;
end;
pos2:=x;
end;{len}
Function subin(pos1,pos2:integer;
               sub:PA;
               pos3,pos4:integer;
               test:PA):Boolean;

{returns true if sub is within test}
var sofar,y,x:integer;
    out,match,fail:boolean;
begin {subin}
out:=false;  y:=pos3;
{the first character in the test string}
REPEAT
  Match:=false;
  While(y<=pos4) and not match do
    begin
    If (sub[pos1]=Test[y]) then match:=true else y:=y+1;
    end;
```

```
  Sofar:=y;
  If not match then begin subin:=false;out:=true;end
{first character of sub is not in test so no match}
 ELSE
 BEGIN
 IF (pos1=pos2) then begin subin:=true;out:=true; end
  ELSE
    Begin
     Sofar:=y; x:=1; fail:=false;
     REPEAT
        If sub[pos1+x]<>test[sofar+x] then fail:=true
        else x:=x+1;
      UNTIL (  (( pos1+x)> pos2) or fail);
      If not fail then begin subin:=true;out:=true;end
      else subin:=false;
    End;
  y:=y+1;
  END;
Until (  (Sofar>=pos4) or (out)  );
END;{subin}
Function len8 (var pos1,pos2:Integer;
                TestString: PA8):Boolean;
Var X         :  integer;
    not_blank:  boolean;
begin
len8:=true;
{FIND REAL LENGTH OF STRING1}
{first find position of first non blank}
not_blank:=false;x:=1;
While (X<=8) and not (not_blank) do
begin
  If (Teststring[x]<>' ')
 Then not_blank:=True Else x:=x+1;
end;
IF (x=9) then len8:=false
ELSE pos1:=x;
{FIND LAST NON BLANK}
x:=8;not_blank:=false;
while(x>=1) and not (not_blank) do
begin
  if (Teststring[x]<>' ') then
  not_blank:= TRUE Else  x:=x-1;
end;
pos2:=x;
end;{len8}
Function subin8(pos1,pos2:integer;
              sub:PA8;
              pos3,pos4:integer;
              test:PA8):Boolean;
{returns true if sub is within test}
var sofar,y,x:integer;
    out,match,fail:boolean;
begin {subin8}
out:=false;  y:=pos3;
{the first character in the test string}
```



```
REPEAT
  Match:=false;
  While(y<=pos4) and not match do
    begin
    If (sub[pos1]=Test[y]) then match:=true else y:=y+1;
    end;
  Sofar:=y;
  If not match then begin subin8:=false;out:=true;end
{first character of sub is not in test so no match}
  ELSE
  BEGIN
    If (pos1=pos2) then begin subin8:=true;out:=true;end
    {if only one character in sub then a match must be a
     successful compare}
    ELSE
    Begin
     Sofar:=y; x:=1; fail:=false;
     REPEAT
        If sub[pos1+x]<>test[sofar+x] then fail:=true
        else x:=x+1;
      UNTIL (  (( pos1+x)> pos2) or fail);
      If not fail then begin subin8:=true;out:=true;end
      else subin8:=false;
    End;
   y:=y+1;
   END;
Until (  (Sofar>=pos4) or (out)  );
END;{subin8}
{a set of procedures for modifying the screen output}
{their names should be self-explanatory              }
Procedure Unline     ;EXTERNAL;
Procedure Bright     ;EXTERNAL;
Procedure Normal     ;EXTERNAL;
Procedure Clearscreen;EXTERNAL;
Procedure Reverse    ;EXTERNAL;
Procedure Bleep      ;EXTERNAL;
procedure revise (var results:rec_of_results);
CONST   Name_length= 40;        {number of characters in
the NAME field}
        Field_length= 8;                {number of
characters in the ID fields}
        Serum_length= 6;                {number of
characters in the serum names}
        Allsera=    240;        {max number of sera in the
sourcefile}
TYPE    Set_of_scores=SET OF CHAR;
VAR     ID_field        :       Array[setoffields]
                                of packed
array[1..detail_length] of char;
        Nowfield        :       Set_of_fields;
        carry_on,
        changed,Found   :       BOOLEAN;
        Eachserum       :       Maxno;
        X,Count         :       Integer;
        Online          :       1..10;
```

```
      detail_correction:        packed
array[1..field_length]OF CHAR;
        NEWname,
        temp_name       :       packed
array[1..name_length]OF CHAR;
        chksrm          :       packed
array[1..serum_length] OF CHAR;
        Pos_scores,
        OK_Scores       :       set_of_scores;
        onechar         :       char;
        screen          :       FILE OF CHAR;
BEGIN
        ID_field[Batch]       :='Batch    ';
        ID_field[Date]        :='Date     ';
        ID_field[sex]         :='sex      ';
        ID_field[Breed]       :='Breed    ';
        ID_field[Location]    :='Location ';
        ID_field[Sublocation]:='Subloctn';
        ID_field[CBTS]        :='CBTS     ';
        ID_field[Spare]       :='Spare    ';
        OK_scores:=['8','6','4','-','2','.','7'];
       Pos_scores:=['8','6','4','7'];
        Assign(Screen,'CON');reset(screen);
{write out the details of the animals id}
write('ANIMAL '); bright; writeln(results.name);normal;
writeln;writeln('DETAILS:-');
for Nowfield:=batch to spare do
write(id_field[Nowfield],' ');
writeln;
writeln('-------------------------------------------------
---------------------------');
for Nowfield:=batch to spare do
write(results.details[Nowfield],' ');
count:=1;
REPEAT
If count=2 then posscores:=['.']
else
begin
  writeln;
  writeln('-------------------------------------------------
-----------------------------');
end;
online:=1;
IF Count=1 then writeln('                          SERA  WITH
SCORES >= 4')
ELSE Writeln            ('
UNTESTED SERA');
For x:=1 to Panel_size do
begin
  If (Results.serums[x] in pos_scores) then
  begin
    If(Results.serums[x]='8') then bright;
    write(serum_panel[x],'  ',Results.serums[x]);
    normal;
    Online:=Online+1;
```



```
    If (online=7) then
    begin
      writeln;
      online:=1;
    end else write('  |  ');
  end;
end;
writeln('--------------');
Count:=count+1;
UNTIL (Count=3);
IF yesno('x','Do you wish to change the ID details') THEN
BEGIN
REPEAT
writeln('--------------');   writeln('name: ',Results.name);
   write  ('enter correct name (<CR> leaves as is) : ');
   FOR X:=1 TO name_length DO NEWname[X]:=' ';
   Count:=1;
   While not eoln(input) and (Count<=namelength) DO
   BEGIN
     read(NEWname[count]);
     NEWname[count]:=caseup(NEWname[count]);
     count:=count+1;
   END;
   readln;
   Count:=1;   changed:=false;
   While (Count<=namelength) and not (changed) do
   BEGIN
     IF NOT (NEWname[count]=' ')THEN Changed:=True;
     Count:=Count+1;
   END;
   IF changed THEN Results.name:=NEWname;
   FOR Nowfield:=Batch TO Spare DO
   BEGIN
      WRITELN(ID_Field[Nowfield],' -
',Results.Details[Nowfield]);
      WRITE  ('Enter correct ',ID_Field[Nowfield],'( <CR>
leaves as is) : ');
      FOR Count:=1 TO 8 DO Detail_correction[Count]:=' ';
{reset the array}
      Count:=1;
      WHILE (NOT eoln(input)) AND (Count<=8) DO
      BEGIN
        READ (Detail_Correction[Count]);
        Detail_correction[Count]:=
       caseup(Detail_correction[count]);
        Count:=Count+1;
      END;       {while not}
      Readln;
      IF NOT (Detail_Correction='         ') THEN {if
correction is not blank}
      Results.Details[Nowfield]:=Detail_Correction;
      {If correction IS blank the field remains unchanged}
   END;  {For Nowfield}
UNTIL (yesno('X','Happy'));
end;
```

```
BEGIN
REPEAT
   FOR Count:=1 TO serum_length DO CHKSRM[Count]:=' ';
   writeln('which serum reaction do you want to check ?:
');
   Count:=1;
   While not(eoln(input)) and (Count<=serum_length) DO
   BEGIN
      Read(chksrm[count]);
      chksrm[count]:=caseup(chksrm[count]);
      Count:=count+1;
   END;
   readln;
   carry_on:=false;
   count:=1;
   repeat
   IF chksrm[count]<>' 'THEN carry_on:=true;
   count:=count+1;
UNTIL  (carry_on) or (count>=serum_length);
IF carry_on THEN
BEGIN
   Eachserum:=0;
   REPEAT
      Eachserum:=Eachserum+1;
   Until (serum_panel[eachserum]=chksrm) OR
(Eachserum>=allsera);
   IF (serum_panel[eachserum]=chksrm) THEN
   BEGIN
      REPEAT
        writeln(serum_panel[eachserum],' reaction is
',results.serums[eachserum]);
        write('enter correct reaction: ');
        onechar:=' ';
        read(screen,onechar); writeln;
        IF  ( onechar=(chr(ord(13))) ) {<CR>}
        THEN onechar:=results.serums[eachserum];
        IF NOT (onechar in OK_scores) THEN bleep;
      UNTIL (onechar IN OK_scores);
      Results.serums[eachserum]:=onechar;
   END
   ELSE Writeln(chksrm,' not found');
END;{if carry on}
UNTIL NOT (carry_on);
END;
end;
BEGIN                          {MAIN PROGRAMME}
Clearscreen;
FOR X:=1 TO 8 Do writeln;
writeln('                  ********************');
writeln('                        Update        ');
writeln('                  ********************');
writeln;writeln;writeln;writeln;writeln;
writeln('sjk 26/10/84');
For X:=1 TO 70000 do;
OK_choice:=['N','T','D','X','B','L','S','C','P','R'];
```



```
Assign(Screen,'CON');  Reset(screen);
        ID_field[Batch]      :='Batch    ';
        ID_field[Date]       :='Date     ';
        ID_field[sex]        :='sex      ';
        ID_field[Breed]      :='Breed    ';
        ID_field[Location]   :='Location';
        ID_field[Sublocation]:='Subloctn';
        ID_field[CBTS]       :='CBTS     ';
        ID_field[Spare]      :='Spare    ';
REPEAT {A LOOP AROUND THE ENTIRE PROGRAM}
Clearscreen;
writeln;writeln;
writeln('This programme allows you to modify the input');
writeln('file. The animals to be modified are chosen in');
writeln('exactly  the same way as the selection program');
writeln('chooses animals to be  copied. USE THE');
writeln('PROGRAMME WITH CARE -  ONLY RUN IT ON  FILES');
writeln('THAT HAVE BEEN   BACKED UP. AN ERROR CAUSED BY');
writeln('POWER FAILURE ETC  CAN DAMAGE THE FILE BEYOND');
writeln('REPAIR.  AFTER "UPDATE" CHECK THE  INTEGRITY');
writeln('OF THE OF THE MODIFIED FILE BY RUNNING IT');
writeln('THROUGH  ANOTHER BoLA PROGRAMME.   Never exit');
writeln('by ^C or ^Break after the input file  has been');
writeln('opened.');
writeln;writeln;
{open the input file}
REPEAT
   write ('Input file: ');
   readln(Infilname);
   IF Not (Fstat(Infilname)) Then writeln('no file
',Infilname);
UNTIL (Fstat(Infilname));
Assign(Infil,Infilname); Update(Infil); writeln;writeln;
{open the batch file}
REPEAT
   write ('Batch file: ');
   readln(Batchfilname);
   IF Not (Fstat(Batchfilname)) Then writeln('no file
',Batchfilname);
UNTIL (Fstat(Batchfilname));
Assign(Batchfil,Batchfilname); Reset(Batchfil);
FOR Count:=1 to allsera do
begin
        For X:=1 to Serum_length do Serum_panel[Count] [X]
:=' ';
end;
FOR Count:=1 to serum_length do select[count]:=' ';
{fill the array serum_panel with the serum names in this
file}
Panel_size:=1;
WHILE (Panel_size<=Allsera) AND (NOT (eof(Batchfil))) DO
  BEGIN
        Count:=1;
        While NOT eoln(Batchfil ) and (Count <=6) DO
                BEGIN
```

```
                READ( Batchfil ,Serum_panel [Panel_size]
[Count] );
                Count:=Count+1;
               End;
        READLN(Batchfil);
        Panel_size:=Panel_size+1;
END;    {while panel size}
Panel_size:=Panel_size-1;
{the serum names are in the array Serum_panel}
{and the variable panel_size is equal to the number of
sera in the panel}
clearscreen;Writeln;Writeln;Writeln;
write('    ');
Writeln('You may select animals for updating by up to
',selectable:1,' strings');
writeln('from one of these fields:-');
writeln;
Writeln;
bright;
Write  ('               N');normal;Writeln('umber');
Write  ('
Ba');bright;write('t');normal;writeln('ch');
bright;
Write  ('               D');normal;writeln('ate ');
normal;
Write  ('               Se');bright;writeln('x');
Write  ('               B');normal;writeln('reed');
Bright;
Write  ('               L');normal;writeln('ocation');
Bright;
Write  ('               S');normal;writeln('ublocation');
Bright;
Write  ('               C');normal;writeln('BTS No    ');
Write  ('
S');Bright;write('p');normal;write('are      ');
Writeln;
writeln;
Write  ('     Or by Serum
');bright;write('r');normal;writeln('eaction ');
writeln;writeln;
writeln('choose the field upon which to base your
selection');writeln;
write('       Choice [',chr(1BH),'[s',{stores cursors
position} ' ]');
repeat
write(chr(1BH),'[u');{return cursor to stored position}
bright;read(screen,choice);normal;
Choice:=Caseup(choice);
Until (Choice IN OK_Choice);
writeln;
IF (Choice='N') Then {select by Name}
Begin
count:=1;   out:=true;
While out and (Count<=selectable) do
begin
```



```
  GoForName:=true;
  Write('Enter id number ',count:1,' to be selected: ');
  For x:=1 to namelength do s_name[count][x]:=' ';
  x:=1;
  While (X<=namelength) and not eoln(input) do
  begin
    read(S_name[count][X]);
    s_name[count][x]:=Caseup(s_name[count][x]);
    x:=x+1;
  end;
readln;
OUT:=(len(posn1[count],posn2[count],s_name[count]) );
{if empty string is entered then leave the loop}
count:=count+1;
end;todo:=count-2;
end {if choice=N}
ELSE {if choice<>N}
Begin
GoForName:=false;   serumonly:=false;
  Case Choice of
  'T':field:=batch;
  'D':field:=date;
  'X':field:=sex;
  'B':field:=breed;
  'L':field:=location;
  'S':field:=sublocation;
  'C':field:=cbts;
  'P':field:=Spare;
  'R':serumonly:=true;
  end;{case}
    IF (not serumonly) then
    Begin
    Count:=1;  out:=true;
      while out and (Count<=selectable) do
      begin
        for x:=1 to detail_length do s_field[count][x]:='
';
        Write('Enter ',ID_Field[field],' ',count:1,' to be
selected: ');
        X:=1;
        While (x<=detail_length) and not eoln(input) do
        Begin
          read(s_field[count][x]);
          s_field[count][x]:=caseup(s_field[count][x]);
          x:=x+1;
        End;
        readln;
        OUT:=(len8(posn1[count],posn2[count],s_field[count])
);
        {until  blank string is entered}
        Count:=Count+1;
      end;todo:=count-2;
    END
  ELSE {selection by serum reaction}
  BEGIN
```

```
{now read the serum_names  to be selected}
x:=1;  out:=false;
WHILE (  NOT (OUT) AND NOT (x>=selectable) ) do
  BEGIN
    Found:=FALSE;
      REPEAT
        FOR Count:=1 TO serum_length DO Select[Count]:='
';
        writeln;
        write('enter the name of serum ',x:1,': ');
        Count:=1;
        While not(eoln(input)) and (Count<=serum_length)
DO
        BEGIN
        read (Select[count]);
        Select[Count]:= caseup( Select[Count] );
        Count:=Count+1;
        END;
      readln;
       IF select<>'       ' then
       begin
{find the position of this serum in the panel}
      Eachserum:=0;
        Repeat
        Eachserum:=Eachserum+1;
        Until  (serum_panel [Eachserum]=select) OR
(Eachserum>=allsera) ;
      IF (serum_panel [Eachserum]=select) THEN Found:=TRUE
      ELSE writeln(select,' not found in ',batchfilname);
      end else out:=TRUE; {if select is empty}
      UNTIL ( (Found) or (out) );
  IF not out then
    BEGIN
    Test[x]:=Eachserum;Opened:=true;
    Repeat
    opened:=true;
    write('select animals pos or neg for ',select,'?  (+/-
) : ');
    read(screen,choice);
     case choice of
     '+':wanted[x]:=true;
     '-':wanted[x]:=false;
     otherwise opened:=false;
     end;{case}
    until (opened);
    x:=x+1;
    END;{if not out}
 END;
todo:=x-1;
writeln;writeln('selection will be by those ',todo:1,'
sera');
writeln;
IF (Yesno('x','count 4 as -ve') )Then Posscores:=['8','6']
ELSE Posscores:=['8','6','4'];
END;
```



```
END;
clearscreen; moved:=0; seen:=0;
writeln;writeln;writeln;
if not serumonly then
begin
  write('                selecting by ',todo:1,' strings in
the ');
  IF goforname then writeln('name') else
writeln(id_field[field],' field');
end
{******  START READING THE FILE ******}
else writeln('                selecting by ',todo:1,' serum
reactions');
continue:=true;
writeln;write('wait');
While not eof(infil) and continue do
begin
ok:=false; seen:=seen+1;
write('.');                                {*****  SELECTION
BY NAME *****}
If GoForName then {if selection is by name}
begin
    If  (len(pos3,pos4,infil^.name) ) then
    {if name from file is not empty}
    begin
      count:=1;
      While (Count<=todo) and not ok do
      begin
        If(subin(posn1[count],posn2[count],s_name[count],
                 pos3,pos4,infil^.name) ) then
        begin
           clearscreen;reverse;
           write('animal number ',seen:4,' in
',infilname,'  '
          ,moved:4,' animal(s) updated - this is number
',(moved+1):4);writeln;
           normal;writeln;
           revise(infil^); moved:=moved+1; ok:=true;
           Put(infil);{write new record to file and go to
next record}
           clearscreen;
           Continue:=(yesno('x','keep searching'));
           If continue then write('wait');
        end; {if subin}
        count:=count+1;
      end; {while count}
    end;  {if len}
    if not ok then Put(infil);{no change has been made-
advance to next record}
END             {if go for name}
Else
begin
IF not serumonly then
{**** SELECTION BY DETAIL FIELD ****}
BEGIN
```

```
  If (len8(pos3,pos4,infil^.details[field]) )then
  begin
    Count:=1; ok:=false;
    While (Count<=todo) and not ok do
    begin
      If (subin8(posn1[count],posn2[count],s_field[count],
                 pos3,pos4,infil^.details[field]) ) then
      begin
           clearscreen;reverse;
           write('animal number ',seen:4,' in
',infilname,'  '
          ,moved:4,' animal(s) updated - this is number
',(moved+1):4);writeln;
           normal;writeln;
           revise(infil^); moved:=moved+1; ok:=true;
           put(infil);
           clearscreen;
           Continue:=(yesno('x','keep searching'));
           If continue then write('wait');
      end;{if subin8}
      Count:=count+1;
    end;  {while count}
   end;    {if len8}
   If not ok then put(infil);
END
ELSE
begin
  {**** SELECTION BY SERUM REACTIONS ****}
  x:=1;
  repeat
    if infil^.serums[test[x]] in pos_scores then
    begin
      if wanted[x] then ok:=true else ok:=false;
    end
    else begin if not wanted[x] then ok:=true else
ok:=false;end;
    x:=x+1;
  until (not (ok) or (x=todo+1));
  if ok then
  begin
      clearscreen;reverse;
      write('animal number ',seen:4,' in ',infilname,'  '
      ,moved:4,' animal(s) updated - this is number
',(moved+1):4);writeln;
      normal;writeln;
      revise(infil^); moved:=moved+1; ok:=true;put(infil);
      clearscreen;
      Continue:=(yesno('x','keep searching'));
      If continue then write('wait');
  end else put(infil);
END;
END;
END;
writeln;
UNTIL (NOT Yesno('X','    make another selection') );
```



```
chain('BoLA');
END.
```

```
                    Re-order serum reactions

PROGRAM switch (INPUT, OUTPUT, INDATAFILE, OUTDATAFILE,
                OLDPANELFILE, NEWPANELFILE);
CONST    ALLSERA           =240;
         NAMELENGTH        =40;
         Serum_length      =6;
         Detail_length     =8;
 TYPE    Maxno             =1..Allsera;
         Set_of_fields
=(batch,date,sex,breed,location,sublocation,
                                      CBTS,Spare);
         Rec_of_results=RECORD
           Name:Packed Array[1..name_length] of char;
        Details:Array[set_of_fields] of packed array
[1..detail_length] of char;
         Serums:Array[maxno] of char;
                         END;
          Snames = Array[Maxno] OF Packed
Array[1..serum_length] OF CHAR;
         BOLAfile          =File Of Rec_of_results;
VAR      Eachserum,Neweachserum,
         Count:            Maxno;
         X:                Integer;
         Results:          Rec_of_results;
         Found,Opened:     BOOLEAN;
         order    :          Array[Maxno] OF integer;
         OldPanel,                  {holds the names of the
old panel sera}
         NewPanel:         Snames;  {holds the names of the
new panel sera}
         Oldpanelfilename,
         Newpanelfilename,
         Indatafilename,
         Outdatafilename:          string[12];
         Indatafile, Outdatafile:          BOLAfile;  {the
pascal non text files}
         Oldpanelfile, Newpanelfile:       TEXT;      {the
two panel files}
FUNCTION YESNO (Default:CHAR;
Message:STRING):BOOLEAN;EXTERNAL;
FUNCTION Caseup (letter: CHAR):CHAR;EXTERNAL;
PROCEDURE Clearscreen;EXTERNAL;
BEGIN
Clearscreen;
Writeln('This program re-orders the reactions in the input
file');
Writeln('so that it coresponds to the NEW PANEL');
{open the file to be added}
REPEAT
   write ('file to be read:');
   readln(Indatafilename);
   IF Not (Fstat(Indatafilename)) Then writeln('no file
',Indatafilename);
UNTIL (Fstat(Indatafilename));
```



```
Assign(Indatafile,Indatafilename); Reset(Indatafile);
{open the TARGET file}
REPEAT
  Opened:=TRUE;
  Write('TARGET file: ');
  Readln(Outdatafilename);
  IF Fstat(Outdatafilename) THEN
     BEGIN
        IF Yesno('X','This file exists OK to append to
it') Then  Opened:=TRUE
     ELSE  Opened:=FALSE;
     END;
UNTIL (Opened);
Assign(Outdatafile,Outdatafilename); Append(Outdatafile);
{open old serum panel file}
REPEAT
   write ('Old serum panel file: ');
   readln(Oldpanelfilename);
   IF Not (Fstat(Oldpanelfilename)) Then writeln('no file
',Oldpanelfilename);
UNTIL (Fstat(Oldpanelfilename));
Assign(Oldpanelfile,Oldpanelfilename);
Reset(Oldpanelfile);
{Open new serum panel file}
REPEAT
   write ('New serum panel file: ');
   readln(Newpanelfilename);
   IF Not (Fstat(Newpanelfilename)) Then writeln('no file
',Newpanelfilename);
UNTIL (Fstat(Newpanelfilename));
Assign(Newpanelfile,Newpanelfilename);
Reset(Newpanelfile);
FOR Count:=1 TO allsera DO
BEGIN
 FOR X:=1 TO serumlength DO
 BEGIN
   Oldpanel[Count][X]:=' ';
   Newpanel[Count][x]:=' ';
 END;
END;
NEWEachserum:=1;
WHILE NOT eof(Newpanelfile) AND (NEWEachserum<=(Allsera-
1)) DO
BEGIN
  Count:=1;
  WHILE NOT EOLN(NEWPANELFILE) AND (COUNT<=SERUM_LENGTH)DO
        BEGIN
          Read(Newpanelfile,Newpanel[NEWEachserum][Count]);
          Newpanel[NEWEachserum][Count]:=
          caseup(Newpanel[NEWEachserum][Count]);
          Count:=Count+1;
        END;
  Readln(Newpanelfile);
  NEWEachserum:=NEWEachserum+1;
END;
```

```
Eachserum:=1;
WHILE NOT eof(Oldpanelfile) AND (Eachserum<=(Allsera-1))
DO
BEGIN
  Count:=1;
  WHILE NOT EOLN(OldpanelFILE) AND (COUNT<=SERUM_LENGTH)DO
        BEGIN
          Read(Oldpanelfile,Oldpanel[Eachserum][Count]);
          Oldpanel[Eachserum][Count]:=caseup(Oldpanel[Each
serum][Count]);
          Count:=Count+1;
        END;
  Readln(Oldpanelfile);
  Eachserum:=Eachserum+1;
END;
For Count:=1 TO NEWEachserum DO
BEGIN
  X:=1; Found:=FALSE;
  WHILE (X<=Eachserum) AND NOT FOUND DO
  BEGIN
    IF Newpanel[Count]=Oldpanel[X] THEN
    BEGIN
     order[Count]:=X;
     Writeln('Well ',Count:3,' is from actual well
',order[Count]:3);
     Found:=TRUE;
    END;
  X:=X+1;
  END;
  IF NOT Found THEN
  BEGIN
    order[Count]:=240; Writeln(Newpanel[Count],' not in
old panel');
  END;
END;
For X:=Neweachserum TO Allsera DO order[X]:=240;
write('wait'); X:=1;
WHILE NOT eof(Indatafile) DO
  BEGIN
  Results:=Indatafile^;  write('.');
  FOR Count:=1 TO Allsera DO Results.Serums[Count]:=
  Indatafile^.serums[order[Count]];
  Write(Outdatafile,Results); get(Indatafile);
  X:=X+1
  END;
Writeln;writeln;
Writeln((x-1):1,' Records copied');
chain('BOLA');
END.
```



```
PROGRAM READ_IN_TENS
(INPUT,OUTPUT,DATAFILE,SERAFILE,RSVFILE,NOTHING);
       CONST allsera=240;      { max number of sera}
             Totplate=40;      { max number of plates}
             Name_length=40;   { length of the name field}
             Kbd_Buffer=15;    { number of characters
                                 in keyboard buffer}
        TYPE One_to_ten=1..10;  One_to_eight=1..8;
             Maxno=1..Allsera;
             Set_of_fields=(batch,date,sex,breed,location,
                            sublocation,CBTS,Spare);
             Set_of_scores=SET OF CHAR;
     Rec_of_Results=RECORD
                      Name:PACKED ARRAY[1..Name_length] OF
CHAR;
                      Details:ARRAY[Set_of_fields] OF
PACKED ARRAY[1..8] OF CHAR;
                      Serums:ARRAY[maxno] OF CHAR;
                      END;
             Allten=ARRAY [One_to_ten] OF Rec_of_Results;
             Field= ARRAY [Set_of_fields] OF PACKED
ARRAY[1..8] OF CHAR;
             Panel= ARRAY [Maxno] OF PACKED ARRAY[1..6] OF
CHAR;
             Allplate=1..Totplate;
             BOLAfile=FILE OF Rec_of_Results;
         VAR Eachanimal,Wrongone:          One_to_ten;
             Results:                      Allten;
             Eachfield:                    Set_of_fields;
             ID_field:                     Field;
             Name_correction:              PACKED
ARRAY[1..Name_length]OF CHAR;
             Detail_correction:            PACKED ARRAY[1..8]
OF CHAR;
             Temp_score_holder:            PACKED ARRAY[1..7]
OF CHAR;
             FirstLook,
             Backcheck,
             Change,Carry_on,
             Opened,Oopsed,Data_ok:        BOOLEAN;
             Serum_panel:                  Panel;
             ASCII,
             X,Count:                      INTEGER;
             Screen,
             Direct:                       FILE OF CHAR;
             Nothing:                      CHAR;
             RSVfile,    {HOLDS the data as it is entered}
             Datafile:   BOLAfile;{holds the data}
             Serafile:   TEXT;{holds the list of sera}
             RSVfname,
             Datafname,
             Serafname:                    STRING[14];
             Set1,Set2:                    STRING[20];
```

```
            Backplate,
            SO_far,Eachplate,Maxplate:  Allplate;
            OKscores:                   Set_of_scores;
            Eachserum,
            Panel_size:                 Maxno;
FUNCTION YESNO (Default:CHAR;
Message:STRING):BOOLEAN;EXTERNAL;
PROCEDURE Bright;EXTERNAL;
PROCEDURE Normal;EXTERNAL;
PROCEDURE Clearscreen;  EXTERNAL;
PROCEDURE Bleep;EXTERNAL;
FUNCTION Caseup (letter: CHAR):CHAR;EXTERNAL;
FUNCTION namecheck(filename:string):Boolean;EXTERNAL;
BEGIN                   {main program}
clearscreen;
FOR X:=1 TO 8 DO writeln;
writeln('                          ***************');
writeln('                          BoLA DATA ENTRY');
writeln('                          ***************');
writeln;writeln;writeln;
writeln('                           by sjk 8-8-84');
FOR X:=1 TO 90000 DO;
Assign(direct,'CON.DIR');reset(direct);{allows input
without holdup}
Assign(screen,'CON');    reset(screen);
        write(chr(1BH),'[0;67;".....";13p');
                            {SETS F9 = ..... <CR> }
        ID_field[Batch]      :='Batch    ';
        ID_field[Date]       :='Date     ';
        ID_field[sex]        :='sex      ';
        ID_field[Breed]      :='Breed    ';
        ID_field[Location]   :='Location ';
        ID_field[Sublocation]:='Subloctn ';
        ID_field[CBTS]       :='CBTS     ';
        ID_field[Spare]      :='Spare    ';
        OKscores:=['-','8','6','4','.','2','7'];
{this set is used for checking serum scores as they are
entered. It contains all the valid scores}
        Clearscreen;
        FOR Eachanimal:=1 TO 10 DO
        {initialise the arrays which will hold}
        {the details of theanimals ID}
        BEGIN
           WITH Results[eachanimal] DO
           BEGIN
        FOR Count:=1 TO Name_length DO Name[Count]:=' ';
              FOR Eachfield:=Batch TO Spare DO
              BEGIN
    FOR Count:=1 TO 8 DO Details[eachfield] [Count]:=' ';
              END;      {For Eachfield}
              {fill the serum fields with A..F}
              {to mark unread plates}
              ASCII:=65;{'A'}
              FOR Eachserum:=1 to allsera  do
              begin
```



```
                SERUMS[Eachserum]:=chr(ord(ASCII));
     If ASCII=70 then ASCII:=65 else ASCII:=ASCII+1;
                end;
           END; {With Results}
         END;   {for eachanimal}
For Count:=1 TO allsera DO                {initialise the
array which will}
BEGIN                                     {hold the serum
names}
       For X:=1 TO 6 DO Serum_panel[Count] [X]:=' ';
END;
{open a file to hold the data  and set it for APPEND}
  REPEAT
    Opened:=true;
    REPEAT
      write('file to hold the data');
      bright;write(' [          ].DTA',chr(1BH),'[15D');
      Readln(Datafname);writeln;normal;
    UNTIL(namecheck(datafname));
    datafname:=concat(datafname,'.DTA');
    IF FSTAT(Datafname) THEN
    BEGIN
    IF Yesno('X','This file exists - ok to append new
data') THEN Opened:=TRUE
                                                          ELSE
Opened:=FALSE
    END;
UNTIL Opened;
Assign(datafile,Datafname); Append(datafile);
{NEXT: open for reading the file holding the names of the
sera}
REPEAT
  REPEAT
    writeln;
    write('file with serum sequence');
    bright;write(' [          ].BCH',chr(1BH),'[15D');
    readln(serafname);writeln;normal;
  UNTIL (namecheck(serafname));
  serafname:=concat(serafname,'.BCH');
  If not (Fstat(serafname)) then writeln('There is no file
',serafname);
UNTIL ( Fstat(serafname) );
Assign(serafile,serafname); reset(serafile);
Panel_size:=0;
WHILE (Panel_size<=Allsera) AND (NOT (eof(Sera_file))) DO
BEGIN
        Panel_size:=Panel_size+1;
        Count:=1;
        While NOT eoln(Sera_file) and (Count <=6) DO
              BEGIN
              READ(Sera_file,Serum_panel[Panel_size][Count]);
              Count:=Count+1;
              End;
        READLN(Sera_file);
END;    {while panel size}
```

```
{the serum names are in the array Serum_panel}
{next calculate the number of plates in the set}
IF (Panel_size DIV 6)=(Panel_size/6) THEN       {if it is
a multiple of 6}
Maxplate:=Panel_size DIV 6
ELSE Maxplate:=((Panel_size DIV 6)+1);
WRITELN('There should be ',maxplate:2,' plates in this
set.' );
WRITELN;
{F7 IS NOW SET TO ANY STRING}
WRITE('ENTER STRING FOR F7: ');READLN(SET1);
SET2:=CONCAT('[0;65;"',SET1,'";13p');
WRITE(CHR(1BH),SET2);
writeln;
IF Yesno('X','Is this the start of a new set of plates')
THEN
BEGIN       {It is a new set - start from the begining}
  SO_far:=1;  {the plate at which to begin reading}
  REPEAT
   Opened:=true;
   write ('file to hold temporary data: ');
   readln(rsvfname);
    IF NOT FSTAT(rsvfname) THEN  assign(rsvfile,rsvfname)
    ELSE
     BEGIN IF (yesno('x','File exists ok to delete it'))
        THEN assign(rsvfile,rsvfname)
        ELSE Opened:=FALSE;
     END;
   UNTIL Opened;
   rewrite(RSVfile);
{open a file to hold data as it is entered }
{    - a safeguard against power cuts!      }
Clearscreen;
FOR Eachanimal:=1 TO 10 DO
BEGIN                    {record animal numbers}
  WRITELN ('ENTER ANIMAL NUMBER ',Eachanimal:2);
  Count:=1;
  WHILE (NOT eoln(input)) AND (Count<=Name_length) DO
  BEGIN
    READ (Results[Eachanimal].Name[Count]);
    Results[Eachanimal].Name[Count]:=
    Caseup(Results[Eachanimal].Name[Count]);       {convert
to upper case}
    Count:=Count+1;
  END;  {while not eoln}
  READLN;
END;                              {numbers recorded}
FOR Eachanimal:=1 TO 10 DO        {fill ID fields}
BEGIN
    Bright;
    Writeln;
    writeln('       Number ',eachanimal:2,'
',results[eachanimal].name);
    normal;
    FOR Eachfield:=Batch TO Spare DO
```



```
    BEGIN
      IF (Eachanimal > 1) THEN
      WRITELN('ANIMAL NUMBER ',Eachanimal:2,': ENTER
',ID_field[eachfield]:8,'    Default is:
',Results[Eachanimal-1].Details[Eachfield])
      ELSE                {this is the 1st animal - no
default}
      WRITELN ('ANIMAL NUMBER ',Eachanimal:2,
             ': ENTER ',ID_field[Eachfield]);
      Count:=1;
      WHILE (NOT eoln(input)) AND (Count<=Name_length) DO
      BEGIN
        READ
(Results[Eachanimal].Details[eachfield][Count]);
 Results[Eachanimal].Details[eachfield][Count]:=
Caseup(Results[Eachanimal].Details[eachfield][Count]);
        Count:=Count+1;
      END;       {while not eoln}
      Readln;
      IF (Eachanimal >1) AND
(Results[Eachanimal].Details[Eachfield]='          ')
      THEN
      Results[Eachanimal].Details[Eachfield]:=
      Results[eachanimal-1].Details[Eachfield];
{ sets each field = to that of the last animal if a null
is entered}
    END;{for eachfield}
END;{for eachanimal}
{ ID details recorded }
For Count:=1 to Kbd_buffer do read(direct,nothing);
{absorbs any extra characters typed in response to above}
{prompts no hold-up if no extra characters}
REPEAT
{now write out the numbers and identities}
   clearscreen;
   FOR Eachanimal:=1 TO 10 DO{write out the names in full}
   BEGIN
     Read(direct,nothing);
     {throw away anything entered while identities are
      being written out}
     WRITE (Eachanimal:2,' - ',Results[Eachanimal].Name);
     IF(results[eachanimal].name[1]='£') THEN
     writeln('**ANIMAL WILL BE IGNORED**')
     ELSE writeln;
   END;
   Writeln;
   WRITELN('ROW   BATCH     DATE     SEX     BREED   LOCTN
SUBLOC    CBTS     SPARE');
   FOR Eachanimal:=1 TO 10 DO
   BEGIN
     WRITE  (Eachanimal:2,' - ');
     FOR Eachfield:=Batch TO Spare DO
     WRITE  ('
',Results[Eachanimal].Details[Eachfield]:8);
     Writeln;
```

```
    END;          { of writing out details }
    writeln;
      IF NOT Yesno('X','DATA OK') THEN   {if data not ok}
      BEGIN                 {correct the error}
      Data_ok:=FALSE;     {there is an error}
      REPEAT
        WRITE('enter number of row to be corrected: ');
        READLN (X);
        UNTIL (X<11) AND (X>0);{until answer is in the
                               legal range of wrongone}
        Wrongone:=X;
        WRITELN('Animal',Wrongone:3,' Number is: ',
        Results[Wrongone].Name);
WRITE  ('Enter correct number (return leaves as is): ');
        FOR Count:=1 TO Name_length DO
Name_correction[Count]:=' '; {reset the array}
        Count:=1;
        WHILE (NOT eoln(input)) AND (Count<=Name_length) DO
        BEGIN
          READ (Name_correction[Count]);
          Name_correction[Count]:=
         Caseup(Name_correction[count]);
          Count:=Count+1;
        END;      {while not}
        Readln;
        Count:=1;
        Change:=FALSE;
        REPEAT
IF NOT (Name_correction[Count]=' ') THEN CHANGE:=TRUE;
           Count:=Count+1;
        UNTIL (Change) OR (Count>=Name_length);
        IF (Change) THEN {if correction is not blank}
     Results[Wrongone].Name:=Name_Correction; {change name}
        {If correction is blank old name remains unchanged}
        FOR Eachfield:=Batch TO Spare DO
        BEGIN
Writeln('Animal:',Wrongone:3,ID_Field[Eachfield]:13,' - ',
                    Results[Wrongone].Details[Eachfield]);
WRITE  ('Enter correct ',ID_Field[Eachfield],': ');
FOR Count:=1 TO 8 DO Detail_correction[Count]:='';
                                  {reset the array}
           Count:=1;
           WHILE (NOT eoln(input)) AND (Count<=8) DO
           BEGIN
             READ (Detail_Correction[Count]);
Detail_correction[Count]:=caseup(Detail_correction[count]);
             Count:=Count+1;
           END;  {while not}
           Readln;
           IF NOT (Detail_Correction='        ') THEN {if
correction is not blank}
           Results[wrongone].Details[Eachfield]:=
          Detail_Correction;
{If correction IS blank the field remains unchanged}
        END;  {For eachfield}
```



```
      END        {if not yesno}
ELSE    {Data is correct}
Data_ok:=TRUE;
UNTIL Data_ok;          {until data is correct}
{Now the information is written into the file RSVfile}
{*****************************************************}
WRITELN('wait this data is being stored in the temporary
file ',rsvfname);
FOR Eachanimal:=1 TO 10 DO
write(RSVfile,Results[Eachanimal]);
CLOSE(RSVfile);
{                          details written to RSVfile     }
END             {if a new set}
ELSE    {it is a part read set -  read the data from the
old RSVfile}
BEGIN
REPEAT
REPEAT
  writeln;
  write('temporary file holding part read test: ');
  readln(rsvfname);
  IF NOT Fstat(rsvfname) THEN Writeln('There is no file
',rsvfname);
UNTIL (Fstat(rsvfname));
Assign(rsvfile,rsvfname);
update(rsvfile);
WRITELN;
WRITELN('This file will continue to be used for temporary
storage');
FOR Eachanimal:=1 TO 10 DO
BEGIN
  Results[Eachanimal]:=RSVfile^;
  get(RSVfile);
END;    {for eachanimal}
CLOSE(RSVfile);
{find how much was read}
Eachplate:=0; Eachserum:=0;
REPEAT
  Eachserum:=Eachserum+6;
  Eachplate:=Eachplate+1;
UNTIL(NOT(Results[1].serums[eachserum] IN OK_Scores));
Eachplate:=eachplate-1;
{now eachplate is the number of plates read}
{write out what has been read from the RSVfile}
   clearscreen;
        WRITELN('ROW   BATCH     DATE      SEX      BREED
LOCTN      SUBLOC     CBTS      SPARE');
        FOR Eachanimal:=1 TO 10 DO
        BEGIN
        WRITELN(' ');
        WRITE  (Eachanimal:2,' - ');
                FOR Eachfield:=Batch TO Spare DO
WRITE  (' ',Results[Eachanimal].Details[Eachfield]:8);
        END;              { of writing out details }
        WRITELN(' ');
```

```
FOR Eachanimal:=1 TO 10 DO  {write out the names in full}
WRITELN (Eachanimal:2,' - ',Results[Eachanimal].Name);
WRITELN;
WRITELN(Eachplate,' Plates read');
UNTIL Yesno
('X','Is this the test you wish to resume reading');
SO_far:=Eachplate+1;
END;   {ELSE}
backcheck:=false;
Clearscreen;
{              BEGIN  READING  SERUM SCORES              }
Oopsed:=false;Data_ok:=true;
Eachplate:=SO_far;
REPEAT
FirstLook:=TRUE;
  Eachanimal:=0;
  REPEAT
   If (not BackCheck) OR (BackCheck and Not FirstLook)
then
   begin
  REPEAT
     Eachanimal:=Eachanimal+1;
        REPEAT
IF  (Results[Eachanimal].name[1]<>'£') THEN
BEGIN
        {program function keys}
write(chr(1BH),'[48;"------";13p'  ); {0  = ------ <CR> }
write(chr(1BH),'[43;13p');            {+  = <CR>        }
        WRITE('Plate:',Eachplate:3,'
Row:',Eachanimal:3,': ');
FOR Count:=1 TO 7 DO Temp_score_holder[Count]:=' ';
{reset the array}
           Oopsed:=FALSE;
           Data_ok:=TRUE;
           Count:=1;
WHILE (NOT eoln(input) AND (Count<=7)) AND (NOT Oopsed) DO
                   BEGIN
                   READ (Temp_score_holder[Count]);
                   IF Temp_score_holder[Count]='£' THEN
Oopsed:=TRUE;
                   Count:=Count+1;
                   END; {While not}
                   READLN;
END
ELSE Temp_score_holder:='...... ';
        IF ((Temp_score_holder[7]<>' ') OR
        (Temp_score_holder[6]=' ')) AND (OOPSED=FALSE)
THEN {if >< 6 scores were recorded}
        BEGIN
           WRITELN('NOT SIX');
           Bleep;
           Data_ok:=FALSE;
        END
        ELSE             {six were entered - check them}
        BEGIN
```



```
        IF NOT Oopsed THEN
        BEGIN
           FOR Count:=1 TO 6 DO
             BEGIN
             IF NOT (Temp_score_holder[Count] IN OKScores)
AND
             (Oopsed=FALSE) THEN
             BEGIN
WRITELN(Temp_score_holder[Count],' is not a legal score');
               Bleep;
               Data_ok:=FALSE;
             END;        {if not in okscores}
           END; {for count}
        END;     {if not oopsed}
        END;     {else}
        UNTIL Data_ok OR OOPSED;
         {if ok write scores to results array}
        IF NOT OOPSED THEN BEGIN
        FOR Count:=1 TO 6 DO
Results[Eachanimal].Serums[((Eachplate-1)*6)+Count]:=
           Temp_score_holder[Count];
        END;     {if not oopsed}
   UNTIL ((Eachanimal>=10) OR OOPSED);
   {reset function keys}
   write(chr(1BH),'[48;48p');       {0  }
   write(chr(1BH),'[43;43p');       {+  }
end;{if not backcheck}
   {NOW CHECK THE DATA FOR EACH PLATE}
   IF NOT Oopsed THEN
   BEGIN
   Clearscreen;
   WRITELN;
   WRITELN;
   WRITELN;
   WRITELN;
   FOR X:=1 TO 6 DO
   BEGIN                  {write out the serum names}
     WRITE('            ');
     FOR Count:=1 TO 6 DO
     WRITE('  ',Serum_panel[((Eachplate-1)*6)+Count][X]);
     WRITE('   ');
     WRITELN;
   END; {for Eachanimal}
   WRITELN;
   WRITELN('          |  A  B  C  D  E  F  |');
   WRITELN('          |--------------------|');
   FOR Eachanimal:=1 TO 10 DO
   BEGIN
     WRITE('      ',Eachanimal:3,' |');
     FOR Count:=1 TO 6 DO
WRITE('  ',
Results[Eachanimal].Serums[((Eachplate-1)*6)+Count]);
     WRITE('  |');
If (Eachanimal=5)then writeln('      PLATE ',eachplate:3)
     Else
```

```
      begin
        If (Eachanimal=6) then writeln('       ----------')
else writeln;
      end;
    END; (for Eachanimal)
    WRITELN('           ----------------------');
    WRITELN;
    WRITELN;
    IF NOT Yesno('X','       Data ok') THEN
    BEGIN
         REPEAT
         If BackCheck then FirstLook:=FALSE;
         WRITE('line at which to begin correcting ?');
         READLN(X);
         UNTIL ( (X<=10) AND (X>0) AND
(results[X].name[1]<>'£') );
{ can't correct a £ animal}
    Eachanimal:=X-1;
   {because Eachanimal will have one added at the start of
the loop}
    Data_ok:=FALSE;
    END  {if not yesno}
    ELSE Data_ok:=TRUE;
   END {if not oopsed}
   ELSE  {was oopsed}
   BEGIN
         REPEAT
           WRITE('line at which to begin correcting ?  (max
is',Eachanimal:3,'):');
           READLN(X);
         UNTIL ( (X<=Eachanimal) AND (X>0) AND
(results[X].name[1]<>'£') );
{must be at or before the line that
was oopsed and not a £ animal}
   Eachanimal:=X-1;
   Data_ok:=FALSE;
   END;
UNTIL (Data_ok);
{data for one plate is correct - store it in RSVfile}
update(RSVfile);
FOR Eachanimal:=1 TO 10 DO
BEGIN
 RSVfile^:=Results[Eachanimal];
 put(RSVfile);
END;     {for eachanimal}
close(RSVfile);
   IF(eachplate<maxplate) AND (So_far<maxplate) then {not
last plate}
   BEGIN
     nothing:=' ';
     write('Carry on ? (y/n/b (=back) ): ');
     read(screen,nothing);writeln;
     case nothing of
       'B','b','Y','y':carry_on:=true;
       'N','n':carry_on:=false;
```



```
       otherwise carry_on:=true;
     end;{CASE}
   END;
   If backcheck then eachplate:=sofar;
{if was backcheck then pick up at plate before backcheck}
   backcheck:=false;
   If Carry_on or (eachplate=maxplate) then
   begin
       Carry_on:=true;{if last plate this allows backcheck}
       If (Nothing='B') or (Nothing='b') or
       (eachplate=maxplate) then
       begin
         If (eachplate=maxplate) then
backcheck:=NOT(Yesno('X','  LAST PLATE - ALL OK'))
         else backcheck:=true;
         If backcheck then
         REPEAT
           write('plate to check: ');readln(backplate);
         UNTIL (backplate>0)and(backplate<=eachplate);
       end;
       If not backcheck then Eachplate:=Eachplate+1
       else
       begin
         sofar:=eachplate;{remember where you got to}
         eachplate:=backplate;
       end;
     end;
     Clearscreen;
UNTIL ( (Eachplate>maxplate) OR (NOT Carry_on ) );
{then fill in the not tested wells maxplate to Totplate}
IF Carry_on THEN         {if got here by finishing the
reading}
BEGIN
         WRITELN('filling in remaining results');
         FOR Eachanimal:=1 TO 10 DO
         BEGIN
             FOR Count:=(((Eachplate-1)*6)+1) TO Allsera DO
             Results[Eachanimal].Serums[Count]:='.';
         END;     {for eachanimal}
{then write all the details to data file}
         FOR Eachanimal:=1 TO 10 DO
         BEGIN
             IF (Results[Eachanimal].name[1]<>'£') THEN
             WRITE(DATAfile,Results[Eachanimal]);
         END; {for eachanimal}
         WRITELN('results written to file ',datafname);
Writeln;
END;        {if not carry on}
write(chr(1BH),'[0;67;0;67p');   {RE-SET F9}
write(chr(1BH),'[0;65;0;65p');   {RE-SET F7}
CHAIN('BOLA');
END.     {main program}
```

```
PROGRAM SELECT
(INPUT,OUTPUT,INFIL,OUTFIL,Rejectfile,batchfile);

CONST   ALLSERA          =240;
        NAMELENGTH       =40;
        Serum_length     =6;
        Detail_length    =8;
        Selectable       =50;  {max number of sera which
can be selected for}
 TYPE   Maxno            =1..Allsera;
        Set_of_fields
=(batch,date,sex,breed,location,sublocation,
                                    CBTS,Spare);
PA              =Packed Array[1..name_length] of char;
PA8             =Packed Array[1..detail_length] of char;
        Rec_of_results=RECORD
          Name:PA;
       Details:Array[set_of_fields] of packed array
[1..detail_length] of char;
        Serums:Array[maxno] of char;
                        END;
        Srmname =          Packed Array[1..serum_length]
OF CHAR;
        BOLAfile         =File Of Rec_of_results;
        ok_scores=       SET OF CHAR;
VAR     seen,todo,
        eachserum,moved,
        Pos3,
        Pos4,Count,X:    integer;
        posn1,posn2:     array[1..selectable] of integer;
        response,
        Choice:          char;
        Field:           Set_of_fields;
        Panel_size:      Maxno;
        Results:         Rec_of_results;
        Exact_match:     Array[1..selectable] of BOOLEAN;
        Reject,
        GoForName,ok,
        Found,serumonly,
        Out,Opened:      BOOLEAN;
        test:            array[1..selectable] of maxno;
        Wanted:          Array[1..selectable] OF Boolean;
        Serum_Panel:     Array[maxno] OF Srmname;{holds
panel sera names}
        Select:          Packed Array[1..serum_length] OF
char;{names of selecting sera}
        Pos_scores:      OK_scores;
        S_name:          array[1..selectable] of PA;
        S_field:         array[1..selectable] of PA8;
                         {as S_name for the id field}
        ID_Field:        Array[set_of_fields] OF STRING[8];
                         {the NAMES of the fields}
        rejectfilname,
```



```
        batchfilname,
        Infilname,
        Outfilname:      string[12];{the names of the files
on disk}
        Screen:          File Of Char;  {gives one chracter
entry without <CR>}
        Batchfil:        TEXT;
        Rejectfile,
        Infil, Outfil:   BOLAfile;  {the pascal non text
files}
        OK_choice:       SET OF 'A'..'Z';
Function len (var pos1,pos2:Integer;
              TestString: PA):Boolean;
{returns positions of first (pos1) and last (pos2)
non blank characters in an array}
Var X         :  integer;
    not_blank:  boolean;
begin
len:=true;
{FIND REAL LENGTH OF STRING1}
{first find position of first non blank}
not_blank:=false;x:=1;
While (X<=40) and not (not_blank) do
begin
  If (Teststring[x]<>' ') Then not_blank:=True Else
x:=x+1;
end;
IF (x=41) then len:=false
ELSE pos1:=x;
{FIND LAST NON BLANK}
x:=40;not_blank:=false;
while(x>=1) and not (not_blank) do
begin
  if (Teststring[x]<>' ') then not_blank:= TRUE Else
x:=x-1;
end;
pos2:=x;
end;{len}
Function subin(pos1,pos2:integer;
               sub:PA;
               pos3,pos4:integer;
               test:PA):Boolean;
{returns true if sub is within test}
var sofar,y,x:integer;
    out,match,fail:boolean;
begin {subin}
out:=false;  y:=pos3;      {the first character in the test
string}
REPEAT
  Match:=false;
  While(y<=pos4) and not match do
    begin
    If (sub[pos1]=Test[y]) then match:=true else y:=y+1;
    end;
  Sofar:=y;
```

```
  If not match then begin subin:=false;out:=true;end
{first character of sub is not in test so no match}
 ELSE
 BEGIN
 IF (pos1=pos2) then begin subin:=true;out:=true; end
  ELSE
    Begin
     Sofar:=y; x:=1; fail:=false;
     REPEAT
        If sub[pos1+x]<>test[sofar+x] then fail:=true
        else x:=x+1;
      UNTIL (  (( pos1+x)> pos2) or fail);
      If not fail then begin subin:=true;out:=true;end
else subin:=false;
    End;
  y:=y+1;
  END;
Until (  (Sofar>=pos4) or (out)  );
END;{subin}
Function match(pos1,pos2:integer;
               sub:PA;
               pos3,pos4:integer;
               test:PA):Boolean;
{returns true if sub matches test ignoring leading and
trailing blanks}
var x:integer;
   matched:boolean;
BEGIN
If (pos2-pos1)<>(pos4-pos3) then match:=false
{if there are not the same number of characters in the 2
strings
then they cannot match}
ELSE
begin
  x:=0; matched:=true; match:=true;
  While (X<=(pos2-pos1) )  and matched do
  begin
    if (sub[pos1+x]<>test[pos3+x]) then matched:=false;
    x:=x+1;
  end;
  match:=matched;
end;
end; {match}
Function match8(pos1,pos2:integer;
               sub:PA8;
               pos3,pos4:integer;
               test:PA8):Boolean;
{returns true if sub matches test ignoring leading and
trailing blanks}
var x:integer;
   matched:boolean;
BEGIN
If (pos2-pos1)<>(pos4-pos3) then match8:=false
{if there are not the same number of characters in the 2
strings
```



```
then they cannot match}
ELSE
begin
  x:=0; matched:=true; match8:=true;
  While (X<=(pos2-pos1) )  and matched do
  begin
    if (sub[pos1+x]<>test[pos3+x]) then matched:=false;
    x:=x+1;
  end;
  match8:=matched;
end;
end; {match}
Function len8 (var pos1,pos2:Integer;
                TestString: PA8):Boolean;
Var X        :  integer;
    not_blank:  boolean;
begin
len8:=true;
{FIND REAL LENGTH OF STRING1}
{first find position of first non blank}
not_blank:=false;x:=1;
While (X<=8) and not (not_blank) do
begin
  If (Teststring[x]<>' ') Then not_blank:=True Else
x:=x+1;
end;
IF (x=9) then len8:=false
ELSE pos1:=x;
{FIND LAST NON BLANK}
x:=8;not_blank:=false;
while(x>=1) and not (not_blank) do
begin
  if (Teststring[x]<>' ') then not_blank:= TRUE Else
x:=x-1;
end;
pos2:=x;
end;{len8}
Function subin8(pos1,pos2:integer;
               sub:PA8;
               pos3,pos4:integer;
               test:PA8):Boolean;
{returns true if sub is within test}
var sofar,y,x:integer;
    out,match,fail:boolean;
begin {subin8}
out:=false;  y:=pos3;     {the first character in the test
string}
REPEAT
  Match:=false;
  While(y<=pos4) and not match do
    begin
    If (sub[pos1]=Test[y]) then match:=true else y:=y+1;
    end;
  Sofar:=y;
  If not match then begin subin8:=false;out:=true;end
```

```
{first character of sub is not in test so no match}
  ELSE
  BEGIN
    If (pos1=pos2) then begin subin8:=true;out:=true;end
    {if only one character in sub then a match must be a
successful compare}
    ELSE
    Begin
     Sofar:=y; x:=1; fail:=false;
     REPEAT
        If sub[pos1+x]<>test[sofar+x] then fail:=true
        else x:=x+1;
      UNTIL (  (( pos1+x)> pos2) or fail);
      If not fail then begin subin8:=true;out:=true;end
else subin8:=false;
    End;
   y:=y+1;
   END;
Until (  (Sofar>=pos4) or (out)  );
END;{subin8}
FUNCTION YESNO (Default:CHAR;
Message:STRING):BOOLEAN;EXTERNAL;
FUNCTION Caseup (letter: CHAR):CHAR;EXTERNAL;
Procedure Unline     ;EXTERNAL;
Procedure Bright     ;EXTERNAL;
Procedure Normal     ;EXTERNAL;
Procedure Clearscreen;EXTERNAL;
Procedure Reverse    ;EXTERNAL;
PROCEDURE WIPE(X:integer); EXTERNAL;
{runs cursor x spaces back down a line erasing as it goes}
BEGIN
Clearscreen;
FOR X:=1 TO 8 Do writeln;
writeln('                ********************');
writeln('                     Selection      ');
writeln('                ********************');
writeln;writeln;writeln;writeln;writeln;
writeln('sjk 6/10/84');
For X:=1 TO 70000 do;
OK_choice:=['N','T','D','X','B','L','S','C','P','R'];
Assign(Screen,'CON');  Reset(screen);
        ID_field[Batch]      :='Batch';
        ID_field[Date]       :='Date';
        ID_field[sex]        :='sex';
        ID_field[Breed]      :='Breed';
        ID_field[Location]   :='Location';
        ID_field[Sublocation]:='Subloctn';
        ID_field[CBTS]       :='CBTS';
        ID_field[Spare]      :='Spare';
REPEAT {A LOOP AROUND THE ENTIRE PROGRAM}
Clearscreen;
writeln;writeln;
Writeln('This program creates an output file by');
Writeln('selecting animals from the  input  file');
Writeln('according  to specified criteria. Note that');
```



```
Writeln('when searching a field a match will be');
Writeln('assumed if the string in the field CONTAINS');
Writeln('the string  being searched for to select only');
Writeln('records which completely match the string');
Writeln('enter the  character"@" before the first');
Writeln('character of the  string Animals not selected');
Writeln('may be written into a REJECT file');
writeln;writeln;
{open the input file}
REPEAT
   write ('Input file: ');
   readln(Infilname);
   IF Not (Fstat(Infilname)) Then writeln('no file
',Infilname);
UNTIL (Fstat(Infilname));
Assign(Infil,Infilname); Reset(Infil);
writeln;
{open the Output file}
REPEAT
  Opened:=TRUE;
  Write('Output file: ');
  Readln(Outfilname);
  IF Fstat(Outfilname) THEN
     BEGIN
        IF Yesno('X','This file exists OK to modify it')
Then  Opened:=TRUE
ELSE  Opened:=FALSE;
     END;
UNTIL (Opened);
Assign(Outfil,Outfilname);
if fstat(outfilname) then
begin
 Repeat
 write('overwrite ',outfilname,' or append to it?
(O/A)');read(screen,choice);
 writeln;
 case choice of
 'a','A':Append(outfil);
 'o','O':rewrite(outfil);
 otherwise opened:=false;
 end;{case}
 until(opened);
 end
else rewrite(outfil);;
{open the batch file}
writeln;
REPEAT
   write ('Batch file: ');
   readln(Batchfilname);
   IF Not (Fstat(Batchfilname)) Then writeln('no file
',Batchfilname);
UNTIL (Fstat(Batchfilname));
Assign(Batchfil,Batchfilname); Reset(Batchfil);
writeln;
Reject:=yesno('X','Save the rejects');
```

```
If reject then
begin
{open the REJECT file}
REPEAT
  Opened:=TRUE;
  Write('Reject file: ');
  Readln(rejectfilname);
  IF Fstat(rejectfilname) THEN
     BEGIN
        IF Yesno('X','This file exists OK to modify it')
Then     Opened:=TRUE
ELSE     Opened:=FALSE;
     END;
UNTIL (Opened);
Assign(rejectfile,rejectfilname);
if fstat(rejectfilname) then
begin
 Repeat
 write('overwrite ',rejectfilname,' or append to it?
(O/A)');read(screen,choice);
 writeln;
 case choice of
 'a','A':Append(rejectfile);
 'o','O':rewrite(rejectfile);
 otherwise opened:=false;
 end;{case}
 until(opened);
 end
else rewrite(rejectfile);;
end;{if reject}
FOR Count:=1 to allsera do
begin
For X:=1 to Serum_length do Serum_panel[Count] [X] :=' ';
end;
FOR Count:=1 to serum_length do select[count]:=' ';
{fill the array serum_panel with the serum names in this
file}
Panel_size:=0;
WHILE (Panel_size<=Allsera) AND (NOT (eof(Batchfil))) DO
  BEGIN
       Panel_size:=Panel_size+1;
       Count:=1;
       While NOT eoln(Batchfil ) and (Count <=6) DO
                BEGIN
READ( Batchfil ,Serum_panel [Panel_size] [Count] );
                 Count:=Count+1;
                End;
       READLN(Batchfil);
END;    {while panel size}
{the serum names are in the array Serum_panel}
{and the variable panel_size is equal to the number of
sera in the panel}
clearscreen;Writeln;Writeln;Writeln;
write('    ');
unline;
```



```
Write('You may select animals by up to ',selectable:1,'
strings from one of these fields:-');
normal;writeln;
Writeln;
bright;
Write  ('                N');normal;Writeln('umber');
Write  ('
Ba');bright;write('t');normal;writeln('ch');
bright;
Write  ('                D');normal;writeln('ate ');
normal;
Write  ('                Se');bright;writeln('x');
Write  ('                B');normal;writeln('reed');
Bright;
Write  ('                L');normal;writeln('ocation');
Bright;
Write  ('                S');normal;writeln('ublocation');
Bright;
Write  ('                C');normal;writeln('BTS No    ');
Write  ('
S');Bright;write('p');normal;write('are      ');
Writeln;
writeln;
Write  ('     Or by Serum
');bright;write('r');normal;writeln('eaction ');
writeln;writeln;
writeln('choose the field upon which to base your
selection');writeln;
write('       Choice [',chr(1BH),'[s',{stores cursors
position} ' ]');
repeat
write(chr(1BH),'[u');{return cursor to stored position}
bright;read(screen,choice);normal;
Choice:=Caseup(choice);
Until (Choice IN OK_Choice);
writeln;
IF (Choice='N') Then {select by Name}
Begin
count:=1;   out:=true;
While out and (Count<=selectable) do
begin
  GoForName:=true;
  Write('Enter id number ',count:1,' to be selected: ');
  For x:=1 to namelength do s_name[count][x]:=' ';
  x:=1;
  read(screen,response);
  {response may be the first character of the string or it
may be the symbol to show that an exact match is wanted}
 If (response<>'@') and (response<>chr(ord(13)) )  then
 begin
    wipe(1);Writeln;
    write(response);
    response:=caseup(response);
    Exact_match[count]:=FALSE;
     x:=2; s_name[count][1]:=response;
```

```
                    {convert response to the first
character of the name}
 end Else
 begin
 write(chr(1BH),'[1D');{backspace}
 if (response<>chr(ord(13))) then begin
     write('  going for an exact match with the string');
     writeln;
     end;
 Exact_match[count]:=TRUE;
    {the symbol "@" shows that an exact match is wanted}
 end;
  If (response=chr(ord(13)) ) then
  begin
     writeln(' END');
     out:=false;x:=44; {<CR> has been entered as first
character so EXIT}
     s_name[count][1]:=' ';
 end else
 begin
  While (X<=namelength) and not eoln(input) do
  begin
    read(S_name[count][X]);
    s_name[count][x]:=Caseup(s_name[count][x]);
    x:=x+1;
  end;
  readln;
 {find position of first and last characters - assigning
to OUT is meaningless}
  OUT:=(len(posn1[count],posn2[count],s_name [count]) );
 end;
count:=count+1;
end;todo:=count-2;
clearscreen;writeln;
writeln('choosing                                    exact
match ?');
for x:=1 to todo do
begin
write(s_name[x],'          ') ;
if (exact_match[x]) then writeln('yes') else
writeln('no');
end;
Write('press any key to continue....');
read(screen,response);
wipe(30);
writeln;
end {if choice=N}
ELSE {if choice<>N}
Begin
GoForName:=false;   serumonly:=false;
  Case Choice of
  'T':field:=batch;
  'D':field:=date;
  'X':field:=sex;
  'B':field:=breed;
```



```
  'L':field:=location;
  'S':field:=sublocation;
  'C':field:=cbts;
  'P':field:=Spare;
  'R':serumonly:=true;
 end;{case}
 If not serumonly then
   Begin
   Count:=1;  out:=true;
     while out and (Count<=selectable) do
     begin
  for x:=1 to detail_length do s_field[count][x]:=' ';
Write('Enter ',ID_Field[field],' ',count:1,
' to be selected: ');
       X:=1;
       read(screen,response);
{response may be the first character of the string or
the symbol "@" to show that an exact match is wanted}
       If (response<>'@') and (response<>chr(ord(13)) )
then
       begin
          wipe(1);writeln;
          write(response);
          response:=caseup(response);
          Exact_match[count]:=FALSE;
          x:=2; s_field[count][1]:=response;
{convert response to the first character of the name}
       end {if response}Else
       begin
         write(chr(1BH),'[1D');{backspace}
         if (response<>chr(ord(13))) then begin
write('  going for an exact match with the string');
                writeln;
                end;
          Exact_match[count]:=TRUE;
{the symbol "@" shows that an exact match is wanted}
       end;{else response}
      If (response=chr(ord(13)) ) then
      begin
        writeln(' END');
       out:=false;x:=44; {<CR> has been entered as first
character so EXIT}
       s_field[count][1]:=' ';
     end {if response}else
     begin
       While (x<=detail_length) and not eoln(input) do
       Begin
         read(s_field[count][x]);
         s_field[count][x]:=caseup(s_field[count][x]);
         x:=x+1;
       End;{while x}
       readln;
OUT:=(len8(posn1[count],posn2[count],s_field[count]) );
       {until  blank string is entered}
       end;{else response is not <CR>}
```

```
      Count:=Count+1;
      end;{ while out}
todo:=count-2;
clearscreen;
writeln;
writeln('choosing                                    exact
match ?');
for x:=1 to todo do
begin
write(s_field[x],'                                   ') ;
if (exact_match[x]) then writeln('yes') else
writeln('no');
end;
Writeln;Write('press any key to continue....');
read(screen,response);
wipe(30);writeln;
  END{if not serumonly}
  ELSE {selection by serum reaction}
  BEGIN
{now read the serum_names  to be selected}
x:=1;  out:=false;
WHILE (  NOT (OUT) AND NOT (x>=selectable) ) do
  BEGIN
    Found:=FALSE;
      REPEAT
FOR Count:=1 TO serum_length DO Select[Count]:=' ';
        writeln;
        write('enter the name of serum ',x:1,': ');
        Count:=1;
  While not(eoln(input)) and (Count<=serum_length) DO
        BEGIN
        read (Select[count]);
        Select[Count]:= caseup( Select[Count] );
        Count:=Count+1;
        END;
      readln;
       IF select<>'       ' then
       begin
{find the position of this serum in the panel}
      Eachserum:=0;
        Repeat
        Eachserum:=Eachserum+1;
        Until  (serum_panel [Eachserum]=select) OR
(Eachserum>=allsera) ;
      IF (serum_panel [Eachserum]=select) THEN Found:=TRUE
      ELSE writeln(select,' not found in ',batchfilname);
      end else out:=TRUE; {if select is empty}
      UNTIL ( (Found) or (out) );
  IF not out then
    BEGIN
    Test[x]:=Eachserum;Opened:=true;
    Repeat
    opened:=true;
write('select animals pos or neg for ',select,'?  (+/-)');
    read(screen,choice);
```



```
    case choice of
    '+':wanted[x]:=true;
    '-':wanted[x]:=false;
    otherwise opened:=false;
    end;{case}
   until (opened);
   x:=x+1;
   END;{if not out}
 END;
todo:=x-1;
writeln;writeln('selection will be by those ',todo:1,'
sera');
writeln;
IF (Yesno('x','count 4 as -ve') )Then Posscores:=['8','6']
ELSE Posscores:=['8','6','4'];
END;
END;
clearscreen; moved:=0; seen:=0;
writeln;writeln;writeln;
if not serumonly then
begin
write('  selecting by ',todo:1,' strings in the ');
  IF goforname then writeln('name') else
writeln(id_field[field],' field');
end
else writeln('selecting by ',todo:1,' serum reactions');
If reject
then writeln('rejects being written to ',rejectfilname);
Write(chr(1BH),'[10;5H','Animals read from ' ,Infilname);
Write(chr(1BH),'[10;45H','Animals written to
',Outfilname);
Reverse;
write(chr(1BH),'[12;15H',seen:1,chr(1BH),'[12;55H',moved:1);
{                                READ THE DATA              }
While not eof(Infil) do
begin
read(Infil,Results);seen:=seen+1;
write(chr(1BH),'[12;15H',seen:1);
If GoForName then {if selection is by name}
   begin
     Count:=1;OK:=false;
     While (Count<=todo) and not OK do
     begin
     If  (len(pos3,pos4,results.name) ) then
     {if name from file is not empty}
       begin
       If( (Exact_match[count]=FALSE)
 and
(subin(posn1[count],posn2[count],
s_name[count],pos3,pos4,results.name)) )
         OR( (Exact_match[Count]=TRUE )
 and (match(posn1[count],posn2[count],
s_name[count],pos3,pos4,results.name)) )
      THEN
          begin
```

```
            write(outfil,results);moved:=moved+1;
            write(chr(1BH),'[12;55H',moved:1);
            OK:=true;
          end; {if subin}
        end;{if len}
        Count:=Count+1;
     end;{while count}
     If not ok and reject then write(rejectfile,results);
     END
Else
begin
IF not serumonly then
BEGIN
  ok:=false;
  If (len8(pos3,pos4,results.details[field]) )then
  begin
    Count:=1;
    While (count<=todo) and not OK do
    begin
      If ((NOT Exact_match[count])
and
(subin8(posn1[count],posn2[count],s_field[count],pos3,pos4
,results.details[field])) )
       OR(    Exact_match[Count]
and
(match8(posn1[count],posn2[count],s_field[count],pos3,pos4
,results.details[field])) )
THEN
      begin
        write(outfil,results);moved:=moved+1;
        write(chr(1BH),'[12;55H',moved:1);
        OK:=true;
      end;{if subin}
      Count:=Count+1;
    end;{while count}
   end;{if len}
   if not ok and reject then write(rejectfile,results);
END
ELSE
begin {select by serum}
x:=1;
repeat
if results.serums[test[x]] in pos_scores then
  begin
  if wanted[x] then ok:=true else ok:=false;
  end
else begin if not wanted[x] then ok:=true else
ok:=false;end;
x:=x+1;
until (not (ok) or (x=todo+1));
if ok then
begin
write(outfil,results);moved:=moved+1;
write(chr(1BH),'[12;55H',moved:1);
end else
```



```
begin
  if reject then write(rejectfile,results);
end;
END;
END;
END;
normal;writeln;writeln;writeln(moved:1,' records copied
into ',outfilname);
UNTIL (NOT Yesno('X','make another selection') );
chain('BoLA');
END.
```

Gene_frequency

```
        Program CHISQ (input,output,currentfile,serafile);

CONST   Allsera      =240;        {max number of sera}
        Serum_length=  6;         {length of serum names}
        Name_length = 40;         {length of animals name}
        Page_length = 50;        {number of lines per page}
TYPE    One_to_ten   =1..10;
        One_to_eight=1.. 8;
        Maxno        =1..Allsera;
        Set_of_fields=
(batch,date,sex,breed,location,sublocation,CBTS,spare);
        Set_of_scores=SET OF CHAR;
        Panel=Array[maxno] of packed
array[1..serum_length] of char;
        Rec_of_results=RECORD
              Name:Packed array[1..Name_length] OF CHAR;
           Details:Array[set_of_fields] OF Packed
array[One_to_eight] of char;
            Serums:Array[Maxno] OF Char;
                   END;
        BOLAfile=FILE OF Rec_of_results;
        TOTALS= Array[1..2] of array [maxno] of integer;
VAR      Results:                 Rec_of_results;
         Test_serum:              packed
array[1..serum_length] of char;
         Serum_panel:             panel;
         validchi,
         loop,X,Count:            Integer;
         Found:                   Boolean;
         Nogo:                    Array[1..2] of boolean;
         Panel_size:              Maxno;
         Posscores:               Set_of_scores;
         PlusScores,
         NegScores,
         NoScores:                TOTALS;
         Seen:                    Array[1..2]of integer;
         Test:                    Array[1..2] of maxno;
         Message,
         workfname,
         Serafname,
         Data2fname,
         Data1fname:              STRING[12];
         Currentfile:             BOLAfile;
         printer,
         Serafile:                TEXT;
         totalchi,
         chisq,
         gf,af,sd:                LONGREAL;
{gene frequency,antigen frequency,standard deviation}
         total:                   Array[1..2] of longreal;
FUNCTION YESNO (Default:CHAR;
Message:STRING):BOOLEAN;EXTERNAL;
Procedure Normal      ;EXTERNAL;
```



```
Procedure Clearscreen;EXTERNAL;
Procedure Reverse     ;EXTERNAL;
FUNCTION Caseup (letter: CHAR):CHAR;EXTERNAL;
FUNCTION calc(total,poss:integer ;var gf:longreal;
                                  var af:longreal;
                                  var
sd:longreal):BOOLEAN;
{A FUNCTION FOR CALCULATING ANTIGEN FREQUENCY, GENE
FREQUENCY AND STANDARD DEVIATION.  IT RETURNS TRUE IF THE
CALCULATION IS  PERFORMED FALSE IF NOT WHEN THE VALUES
RETURNED ARE ZERO, THIS HAPPENS IF TOTAL  <= 0 TO AVOID A
DIVIDE BY ZERO ERROR AND IF POSS=TOTAL TO PREVENT A SQ
ROOT OF ZERO ERROR}
var lrposs,lrtotal:longreal;
begin
      calc:=true;
      IF(total>0) AND (total>poss) then
      begin
        lrposs:=poss;lrtotal:=total;
        af:=poss/total;
        gf:=(1-(sqrt(1-(poss/total))));
        sd:=sqrt(gf/(2*total));
      end
      else begin calc:=false; af:=0; gf:=0; sd:=0; end;
end;{calc}
Function calcchisq(pos1,pos2,neg1,neg2:integer;var
chisq:longreal):boolean;
{a function for calculating chi-square it returns TRUE if
the calculation was performed and FALSE if it was not
(because an expected value is <=1)}
in which case the chi-sq returned is zero}
VAR expos1,expos2,exneg1,exneg2,total1,total2:longreal;
BEGIN
calcchisq:=true;
total1:=pos1+neg1;
total2:=pos2+neg2;
IF (total1>0) and (total2>0) then
  begin
  expos1:=
  ((pos1+pos2) / (total1+total2))*total1;
  expos2:=
  ((pos1+pos2) / (total1+total2))*total2;
  exneg1:=
  ((neg1+neg2) / (total1+total2))*total1;
  exneg2:=
  ((neg1+neg2) / (total1+total2))*total2;
{now calculate chi-sq}
  If (expos1>1) and (expos2>1) and (exneg1>1) and
(exneg2>1) then
    chisq:=
     (sqr(pos1-expos1) / expos1) +
     (sqr(pos2-expos2) / expos2) +
     (sqr(neg1-exneg1) / exneg1) +
     (sqr(neg2-exneg2) / exneg2)
    else begin chisq:=0;calcchisq:=false;end;
```

```
end else begin chisq:=0;calcchisq:=false;end;
end;{calcchisq}
BEGIN
clearscreen;
FOR X:=1 TO 8 Do writeln;
writeln('                    ********************');
writeln('                         Chi-square     ');
writeln('                    ********************');
writeln;writeln;writeln;writeln;writeln;
writeln('sjk 11/10/84');
For X:=1 TO 60000 do;
REPEAT {a loop around the whole program}
clearscreen;
FOR Count:=1 to allsera do
begin
For X:=1 to Serum_length do Serum_panel[Count] [X] :=' ';
end;
{now open the files with the data}
REPEAT
  write('First File with BoLA data: ');
  readln(data1fname);
  IF NOT Fstat(data1fname) THEN writeln ('There is no file
',data1fname);
Until (Fstat(data1fname));
REPEAT
  write('Second File with BoLA data: ');
  readln(data2fname);
  IF NOT Fstat(data2fname) THEN writeln ('There is no file
',data2fname);
Until (Fstat(data2fname));
{open the file holding the serum panel}
REPEAT
  Write('File with serum panel: ');
  readln(serafname);
  IF Not Fstat(serafname) Then writeln ('There is no file
',serafname);
UNTIL (Fstat(serafname));
Assign(serafile,serafname); reset(serafile); writeln;
assign(printer,'prn');rewrite(printer);
IF (Yesno('x','count 4 as -ve') )Then Posscores:=['8','6']
ELSE Posscores:=['8','6','4'];
{fill the array serum_panel with the serum names}
Panel_size:=0;
WHILE (Panel_size<=Allsera) AND (NOT (eof(Sera_file))) DO
  BEGIN
       Panel_size:=Panel_size+1;
       Count:=1;
       While NOT eoln(Sera_file) and (Count <=6) DO
                BEGIN
READ( Sera_file,Serum_panel [Panel_size] [Count] );
                 Count:=Count+1;
                End;
       READLN(Sera_file);
END;    {while panel size}
{the serum names are in the array Serum_panel}
```



```
{and the variable panel_size is equal to the number of
sera in the panel}
{initialise th arrays holding the counts}
For loop:=1 to 2 do
begin
   For count:=1 to allsera do
   begin
   PlusScores[loop] [count]:=0;
    NegScores[loop] [count]:=0;
    NoScores [loop] [count]:=0;
   end;
end;
clearscreen;
seen[1]:=0; seen[2]:=0;
Write(chr(1BH),'[10;5H', 'Animals read from '
,data1fname);
Write(chr(1BH),'[10;45H','Animals read from ',data2fname);
Reverse;
write(
chr(1BH),'[12;15H',seen[1]:1,chr(1BH),'[12;55H',seen[2]:1);
{**loop to look at each file**}
For loop:=1 to 2 do
begin
  If (loop=1) then Assign(currentfile,data1fname)
  else             Assign(currentfile,data2fname);
  reset(currentfile);
  {**loop to look at each animal in the file**}
  While not eof(currentfile) do
  begin
    read(currentfile,results);
    IF (results.name[1]<>'£') THEN
    BEGIN
       seen[loop]:=seen[loop]+1;
       IF loop=1 then write(chr(1BH),'[12;15H',seen[1]:1)
else
       write(chr(1BH),'[12;55H',seen[2]:1);
       {**loop to look at each serum of one animal**}
       For Count:=1 to panel_size do
       begin
          IF NOT (Results.serums[Count]='.') THEN
          BEGIN
            IF Results.serums[Count] IN Posscores Then
PlusScores[loop] [count]:=PlusScores[loop] [count]+1
Else NegScores[loop] [count]:=NegScores[loop] [count]+1;
          END
ELSE    NoScores[loop] [count]:= NoScores[loop] [count]+1;
       end;{for count}
    end;{if not £}
  end;{while not eof}
end;{for loop}
writeln(printer,chr(0CH));{form feed}
write(printer,chr(1BH),'!',chr(ord(8)) );
{set print style 8}
IF ('4'IN Posscores)THEN
     Write(printer,'  4 is considered +ve',chr(0DH))
```

```
ELSE write(printer,'  4 is considered -ve',chr(0DH));
writeln(printer);writeln(printer);
 write(printer,data1fname,' containing ',seen[1]:5,'
animals',
'             ',data2fname,' containing ',seen[2]:5,'
animals',chr(0DH));
writeln(printer,'_____________________________________
_____________________________________');
writeln(printer);
writeln(printer);
write(printer,'SERUM');
for x:=1 to 2 do
write(printer,'    agen freq(%) gene freq  SD   ');
writeln(printer,'          CHI SQ     Significant ?');
writeln(printer);
totalchi:=0; validchi:=0;
For Count:=1 to panel_size do
begin
  Write(printer,Serum_panel[Count],'  ');
  For loop:=1 to 2 do
  begin
   IF(calc(
(plusscores[loop][count]+negscores[loop][count]),
            plusscores[loop][count],gf,af,sd)) then
       write(printer,100*af:10:2,gf:10:4,sd:10:4,'  ;  ')
   else
       write(printer,'cannot do',
(plusscores[loop][count]+negscores[loop][count]):5 ,
' tested',plusscores[loop][count]:5,' +ve     ');
  end;  {for loop}
  if(calcchisq(plusscores[1][count],plusscores[2][count],
  negscores[1][count], negscores[2][count],chisq))
  then
  begin
    validchi:=validchi+1;  totalchi:=totalchi+chisq;
    If chisq>10.83 then
write(printer,chr(1BH),'!',chr(ord(24)) );
{set print style 24}
    write(printer,'   ',chisq:10:2);
    IF chisq>2.7 then
       begin
IF not (chisq>3.84) then
writeln(printer,'     P=10 % *') else
         begin
IF not (chisq>6.63) then writeln(printer,'     P=5
% **') else
           begin
IF not (chisq>10.83) then writeln(printer,'     P=1
% ***')else
                                writeln(printer,'
P<0.1% ****',chr(1BH),'!',chr(ord(8)) );
            end;
          end;
        end else  writeln(printer,'     NOT SIG');
   end
```



```
   else writeln(printer,'    an expected value <= 1');
end;{for count}
writeln(printer);
writeln(printer,'                          ',validchi:5,
            ' CHI SQ CALCULATIONS WERE MADE  - THE SUM OF
THESE CHI SQs IS ',
           totalchi:10:4);
writeln(printer,'                                MEAN
IS',totalchi/validchi:10:4);
normal;writeln;writeln;
UNTIL NOT (Yesno('X','Make another chi square comparison'));
Chain('BoLA');
END.
```

```
PROGRAM cluster (INPUT, OUTPUT, INDATAFILE, OUTDATAFILE,
                 OLDPANELFILE, NEWPANELFILE,clsFILE);

CONST    Allsera        =240;
         NAME_LENGTH     =40;
         Serum_length   =6;
         Detail_length  =8;
         Maxclstr=       40;
         maxperclstr=    40;
 TYPE    Maxno=1..Allsera;
         Set_of_fields
=(batch,date,sex,breed,location,sublocation,
                                        CBTS,Spare);
         Rec_of_results=RECORD
           Name:Packed Array[1..name_length] of char;
        Details:Array[set_of_fields] of packed array
[1..detail_length] of char;
         Serums:Array[maxno] of char;
                        END;
          Snames = Array[Maxno] OF Packed
Array[1..serum_length] OF CHAR;
         BOLAfile         =File Of Rec_of_results;
VAR      Clstrname:       Array[1..maxclstr] of Packed
Array[1..serum_length] of char;
         Panelsera:       Array[1..Allsera] of packed
array[1..6] of char;
         Eachclstr:       1..maxclstr;
         Number_in_batch,
         Eachserum,
         Count,X:         maxno;
         Temp:            packed array[1..serum_length] of
char;
         Totalclstrs:   1..maxclstr;
         Totalsera:       Array [1..maxclstr] of 1..maxclstr;
         Results:         Rec_of_results;
         Out     :        Array[1..2] of boolean;
         Found,Opened:    BOOLEAN;
         order    :       Array[1..Maxclstr] OF
array[1..maxperclstr] of maxno;
         Pos_scores:      SET OF CHAR;
         Choice:          CHAR;
         Clsfilename,
         Oldpanelfilename,
         Newpanelfilename,
         Indatafilename,
         Outdatafilename:string[12];
          Indatafile, Outdatafile:        BOLAfile;
   {the pascal non text files}
          Clsfile,
   {the file with cluster details}
          Oldpanelfile, Newpanelfile:     TEXT;
   {the two panel files}
FUNCTION YESNO (Default:CHAR;
```



```
Message:STRING):BOOLEAN;EXTERNAL;
PROCEDURE Clearscreen;  EXTERNAL;
PROCEDURE Bleep;        EXTERNAL;
FUNCTION Caseup (letter: CHAR):CHAR;EXTERNAL;
BEGIN
Clearscreen;
Writeln('This program adds reactions to the animals in the
input file');
Writeln('corresponding to the presence or absence of
clusters');
writeln('defined in the CONTROL file');
REPEAT
writeln;
write('Input File: ');
readln(Indatafilename);
IF NOT Fstat(Indatafilename) THEN Writeln('There is no
file ',Indatafilename);
UNTIL (Fstat(Indatafilename));
Assign(Indatafile,Indatafilename);
reset (Indatafile);
REPEAT
writeln;
write('file with serum names(the batch): ');
readln(oldpanelfilename);
IF NOT Fstat(oldpanelfilename) THEN
Writeln('There is no file ',oldpanelfilename);
UNTIL (Fstat(oldpanelfilename));
Assign(oldpanelfile,oldpanelfilename);
reset(oldpanelfile);
REPEAT
writeln;
write('file with clustering data ("CON" = keyboard): ');
readln(clsfilename);
If not ( (clsfilename='con') or (clsfilename='CON') ) then
begin
  IF NOT Fstat(clsfilename) THEN Writeln('There is no file
',clsfilename);
end else clsfilename:='CON';
{changes lower case to upper case}
UNTIL ( (Fstat(clsfilename)) or (clsfilename='CON') );
Assign(clsfile,clsfilename);
reset(clsfile);
{open the Output file}
REPEAT
  WRITELN;
  Opened:=TRUE;
  Write('Output file: ');
  Readln(Outdatafilename);
  IF Fstat(Outdatafilename) THEN
     BEGIN
        IF Yesno('X','This file exists OK to modify it')
Then  Opened:=TRUE
ELSE  Opened:=FALSE;
     END;
UNTIL (Opened);
```

```
Assign(outdatafile,Outdatafilename);
if fstat(Outdatafilename) then
begin
  Repeat
write('overwrite ',Outdatafilename,' or append ? (O/A)');
     readln(choice);
     writeln;
     case choice of
     'a','A':Append(outdatafile);
     'o','O':rewrite(outdatafile);
     otherwise opened:=false;
     end;{case}
   until(opened);
 end
else rewrite(outdatafile);;
{open the Output file}
REPEAT
  Opened:=TRUE;
  WRITELN;
  Write('File for new batch file: ');
  Readln(newpanelfilename);
  IF Fstat(newpanelfilename) THEN
     BEGIN
        IF Yesno('X','This file exists OK to delete it')
Then Opened:=TRUE
ELSE Opened:=FALSE;
     END;
UNTIL (Opened);
Assign(Newpanelfile,Newpanelfilename);
Rewrite(Newpanelfile);
FOR Count:=1 TO allsera DO
BEGIN
 FOR X:=1 TO serum_length DO
   Panelsera[Count][X]:=' ';
END;
{ fill the array Panel_sera with the names of the sera}
Eachserum:=1;
WHILE NOT eof(Oldpanelfile) AND (Eachserum<=(Allsera-1))
DO
BEGIN
  Count:=1;
  WHILE NOT EOLN(OldpanelFILE) AND (COUNT<=SERUM_LENGTH)DO
        BEGIN
          Read(Oldpanelfile,Panelsera[Eachserum][Count]);
          Panel_sera[Eachserum][Count]:=
          Caseup(Panel_sera[Eachserum][Count]);
          Count:=Count+1;
        END;
        Readln(Oldpanelfile);
        Eachserum:=Eachserum+1;
END;
number_in_batch:=eachserum-1;
For count:=1 to maxclstr do
begin
  for x:=1 to serum_length do clstrname[count][x]:=' ';
```



```
end;
Pos_scores:=['8','6'];
Eachclstr:=1; out[1]:=false;
while (eachclstr<=maxclstr) and not out[1] do
begin
  writeln;
  If (clsfilename='CON') then{if reading from keyboard}
  write('Enter name of cluster ',eachclstr:1,': ');
  x:=1;
  while not eoln(clsfile) and (x<=serum_length) do
  begin
    read(clsfile,clstrname[eachclstr][x]);
    x:=x+1;
  end;
  readln(clsfile);
  If (clstrname[eachclstr]<>'       ') then
  begin
    Eachserum:=1; out[2]:=false;
    While (Eachserum<=maxperclstr) and not out[2] do
    begin
      REPEAT
        If (clsfilename='CON')then
{if reading from keyboard}
        Write('Enter name of serum ',Eachserum:1,
              ' of cluster ',clstrname[eachclstr]);
        For x:=1 to serum_length do temp[x]:=' ';
        x:=1;
        While not eoln(clsfile) and (x<=serum_length) do
        begin
          read(clsfile,temp[x]);
          Temp[X]:=Caseup(Temp[x]);
          x:=x+1;
        end;
        readln(clsfile);
        If not (temp='       ') then
        begin
      {find the position of this serum in the panel}
          X:=1; found:=false;
          While (x<=number_in_batch) and not found do
          begin
            If (temp=Panel_sera[x]) then
            begin
              found:=true;
              order[eachclstr][eachserum]:=x;
            end;
            x:=x+1;
          end;
          If not found then writeln(temp,' not found');
        end else
        begin
          Out[2]:=true;
          totalsera[eachclstr]:=eachserum-1;
        end;
        Until( (found) or out[2]);
        Eachserum:=eachserum+1;
```

```
      end;
    end else
    begin
      out[1]:=true;
      totalclstrs:=eachclstr-1;
    end;
    eachclstr:=eachclstr+1;
  end;
  writeln(totalclstrs:1,' clusters');
  writeln;
  for x:=1 to totalclstrs do
  begin
    writeln(clstrname[x],' has ',totalsera[x],' sera');
  end;
{now create the new batch file}
For x:=1 to number_in_batch do
writeln(newpanelfile,Panel_sera[x]);
for x:=1 to totalclstrs     do
writeln(newpanelfile,clstrname[x]);
{                          NOW  READ  THE  DATA FILE   }
writeln;write('Wait');
While not eof(indatafile) do
begin
  Read(indatafile,Results);
  For Eachclstr:=1 to totalclstrs do
  begin
    Eachserum:=1;  Out[1]:=false;
While (Eachserum<=totalsera[eachclstr]) and not out[1] do
    begin
      If not (Results.serums[order[eachclstr][eachserum] ]
in pos_scores) then
      out[1]:=true;
      Eachserum:=eachserum+1;
    end; {while eachserum}
    If not out[1] then
Results.serums[number_in_batch+eachclstr]:='8'
                  Else
Results.serums[number_in_batch+eachclstr]:='-';
  end;{for eachclstr}
  Write(outdatafile,Results);write('.')
end;{while not eof}
WRITELN('done');
Chain('BoLA');
end.
```



```
PROGRAM FORPAS (INPUT,OUTPUT,Sourcefil,Targetfil);
CONST   ALLSERA          =240;
        NAMELENGTH       =40;
        Serum_length     =6;
        Detail_length    =8;
 TYPE   Maxno            =1..Allsera;
        Set_of_fields
=(batch,date,sex,breed,location,sublocation,
                                      CBTS,Spare);
        Rec_of_results=RECORD
          Name:Packed Array[1..name_length] of char;
       Details:Array[set_of_fields] of packed array
[1..detail_length] of char;
        Serums:Array[maxno] of char;
                        END;
        BOLAfile        =File Of Rec_of_results;
VAR     X,Copy :        integer;
        Opened:         BOOLEAN;
        Targetfilname,
        Sourcefilname:  string[12];
        Sourcefil, Targetfil:   BOLAfile;
{the pascal non text files}
FUNCTION YESNO (Default:CHAR;
Message:STRING):BOOLEAN;EXTERNAL;
FUNCTION Caseup (letter: CHAR):CHAR;EXTERNAL;
PROCEDURE Clearscreen;EXTERNAL;
BEGIN
Clearscreen;
writeln('This program merges files written in the');
writeln('PASCAL format. Data is read from the SOURCE');
writeln('file and appended to the contents of the');
writeln('TARGET file.  If the TARGET file does not');
writeln('exist a new file is opened');
writeln;{open the file to be added}
REPEAT
   write ('SOURCE file: ');
   readln(Sourcefilname);
   IF Not (Fstat(Sourcefilname))
Then writeln('no file ',Sourcefilname);
UNTIL (Fstat(Sourcefilname));
Assign(Sourcefil,Sourcefilname); Reset(Sourcefil);
{open the TARGET file}
REPEAT
  Opened:=TRUE;
  writeln;
  Write('TARGET file: ');
  Readln(Targetfilname);
  IF Fstat(Targetfilname) THEN
     BEGIN
        IF Yesno('X','This file exists OK to append to it')
     Then Opened:=TRUE
     ELSE Opened:=FALSE;
     END;
```

```
UNTIL (Opened);
Assign(Targetfil,Targetfilname); Append(Targetfil);
writeln;writeln('How many animals to copy ?: ');
writeln('This number EXCLUDES "deleted" records which will
not be copied');
write('Number: '); readln(copy);
X:=1; writeln; write('wait');
{***************** READ THE FILES }
WHILE NOT eof(Sourcefil) and (x<=copy) DO
BEGIN
  IF (Sourcefil^.name[1]<>'£') Then
  begin
    Targetfil^:=Sourcefil^;
    x:=x+1; write('.');
    Put(Targetfil); Get(Sourcefil);
  End else get(sourcefil);
END;
Writeln;
Writeln((x-1):1,' Records copied');
chain('BOLA');
END.
```


Convert_Pascal_to_FORTRAN

```
PROGRAM Pascal_Fortran (INPUT,OUTPUT,FORFIL,PASFIL);
CONST    ALLSERA          =240;
         NAMELENGTH       =40;
         Serum_length     =6;
         Detail_length    =8;
 TYPE    Maxno            =1..Allsera;
         Set_of_fields
=(batch,date,sex,breed,location,sublocation,
                                      CBTS,Spare);
         Rec_of_results=RECORD
           Name:Packed Array[1..name_length] of char;
        Details:Array[set_of_fields] of packed array
[1..detail_length] of char;
         Serums:Array[maxno] of char;
                          END;
         BOLAfile         =File Of Rec_of_results;
VAR      X,Count,Copy:    integer;
         Results:         Rec_of_results;
         Eachfield:       Set_of_fields;
         Opened:          BOOLEAN;
         Pasfilname,
         Forfilname:      string[12];
         Pasfil:          BOLAfile;
{the pascal non text file}
         Forfil:          TEXT;
{the fortran text file}
FUNCTION YESNO (Default:CHAR;
Message:STRING):BOOLEAN;EXTERNAL;
FUNCTION Caseup (letter: CHAR):CHAR;EXTERNAL;
PROCEDURE Clearscreen;EXTERNAL;
BEGIN
Writeln('This program converts data files from the');
Writeln('PASCAL formatto the FORTRAN format');
{open the PASCAL file}
REPEAT
   write ('PASCAL file: ');
   readln(pasfilname);
   IF Not (Fstat(pasfilname)) Then writeln('no file
',pasfilname);
UNTIL (Fstat(pasfilname));
Assign(Pasfil,pasfilname); Reset(pasfil);
{open the FORTRAN file}
REPEAT
  Opened:=TRUE;
  Write('FORTRAN file: ');
  Readln(Forfilname);
  IF Fstat(Forfilname) THEN
     BEGIN
        IF Yesno('X','This file exists OK to erase it')
Then Opened:=TRUE
ELSE Opened:=FALSE;
     END;
UNTIL (Opened);
```

```
Assign(Forfil,Forfilname); Rewrite(Forfil);
writeln;
write('How many animals do you want to be copied ?: ');
readln(copy);
X:=1;
WHILE NOT eof(Pasfil) and (x<=copy) DO
  BEGIN
  Read(Pasfil,Results);
            Writeln(Forfil,Results.Name);
            For Eachfield:=Batch to Spare do
                Write(Forfil,Results.Details[Eachfield] );
            writeln(Forfil);
            For Count:=1 TO 120  DO
Write(Forfil,Results.Serums[Count]);
            Writeln(Forfil);
            For Count:=121 TO Allsera DO
Write(Forfil,Results.Serums[Count]);
            Writeln(Forfil);
IF (X/10=X div 10) then writeln(X:1,' copied');
            x:=x+1;
END;
Writeln;
Writeln((x-1):1,' Records copied');
chain('BOLA');
END.
```


Convert Fortran data to Pascal data

```
PROGRAM FORPAS (INPUT,OUTPUT,FORFIL,PASFIL);
CONST   ALLSERA          =240;
        NAMELENGTH       =40;
        Serum_length     =6;
        Detail_length    =8;
 TYPE   Maxno            =1..Allsera;
        Set_of_fields
=(batch,date,sex,breed,location,sublocation,
                                      CBTS,Spare);
        Rec_of_results=RECORD
          Name:Packed Array[1..name_length] of char;
       Details:Array[set_of_fields] of packed array
[1..detail_length] of char;
        Serums:Array[maxno] of char;
                         END;
        BOLAfile         =File Of Rec_of_results;
VAR     X,Count,Copy:    integer;
        Results:         Rec_of_results;
        Eachfield:       Set_of_fields;
        Opened:          BOOLEAN;
        Pasfilname,
        Forfilname:      string[12];
        Pasfil:          BOLAfile;
{the pascal non text file}
        Forfil:          TEXT;
{the fortran text file}
FUNCTION YESNO (Default:CHAR;
Message:STRING):BOOLEAN;EXTERNAL;
FUNCTION Caseup (letter: CHAR):CHAR;EXTERNAL;
PROCEDURE Clearscreen;EXTERNAL;
BEGIN
Clearscreen;
Writeln('This program converts data files from the');
Writeln('FORTRAN format to the PASCAL format');
{open the FORTRAN file}
REPEAT
   write ('FORTRAN file: ');
   readln(forfilname);
   IF Not (Fstat(forfilname)) Then
writeln('no file ',forfilname);
UNTIL (Fstat(forfilname));
Assign(Forfil,Forfilname); Reset(forfil);
{open the PASCAL file}
REPEAT
  Opened:=TRUE;
  Write('PASCAL file: ');
  Readln(Pasfilname);
  IF Fstat(Pasfilname) THEN
     BEGIN
        IF Yesno('X','This file exists OK to erase it')
Then Opened:=TRUE
ELSE Opened:=FALSE;
     END;
```

```
UNTIL (Opened);
Assign(Pasfil,Pasfilname); Rewrite(Pasfil);
writeln;write('How many animals to be moved ?:');
readln(copy);
X:=1;
WHILE NOT eof(Forfil) and (X<=Copy) DO
  BEGIN
        {reset the arrays holding name & details}
    For Count:=1 TO Namelength do
Results.Name[Count]:=' ';
    FOR Count:=1 TO Detail_length do
Results.Details[Spare] [Count]:=' ';
    Count:=1;
    While not (eoln(Forfil)) AND (Count<=Namelength) DO
        BEGIN
            Read(Forfil,Results.Name[Count]);
            Results.Name[Count]:=Caseup(Results.Name[Count]);
            Count:=Count+1;
        END;
    Readln(forfil);
    For Eachfield:=Batch to CBTS do
        BEGIN
        For Count:=1 TO Detail_length do
           BEGIN
Read(Forfil,Results.Details[Eachfield] [Count]);
                Results.Details[Eachfield] [Count]:=Caseup
               (Results.Details[Eachfield] [Count]);
           END;
        END;
     Count:=1;
While not (eoln(Forfil)) And (Count<=Detail_length) DO
     BEGIN
        Read (Forfil,Results.Details[Spare] [Count]);
        Results.Details[Spare] [Count]:=Caseup
       (Results.details[Spare] [Count]);
        Count:=Count+1;
     END;
     Readln(Forfil);
     For Count:=1 TO 120       DO
Read(Forfil,Results.Serums[Count]);
     Readln(Forfil);
     For Count:=121 TO Allsera DO
Read(Forfil,Results.Serums[Count]);
     Readln(Forfil);
     IF (X/10=X div 10) then writeln(X:1,' copied');
     X:=X+1;
Write(Pasfil,Results);
END;
Writeln;
Writeln((x-1):1,' Records copied');
chain('bola');
END.
```



```
SEGMENT KEMPLIB;

procedure pasdate(VAR year,day:INTEGER;VAR ms:STRING);
{extracts the system date and converts the month to a 3
character name}
{the integer month from the system is held in MONTH}
{the character month is held in string MS}

VAR month:INTEGER;
procedure    date(VAR year,day,month:integer);external;
{"date" is the PROSPERO date procedure}
begin{pasdate}
date(year,day,month);
case month of
 1:ms:='JAN'; 2:ms:='FEB'; 3:ms:='MAR'; 4:ms:='APR';
 5:ms:='MAY'; 6:ms:='JUN'; 7:ms:='JLY'; 8:ms:='AUG';
 9:ms:='SEP';10:ms:='OCT';11:ms:='NOV';12:ms:='DEC';
End;{case}
end;{pasdate}

FUNCTION YESNO (Default:CHAR; Message:STRING):BOOLEAN;
{ a routine which returns true if y OR Y are entered}
{ returns false if n or N are entered}
{ the default may be defined as true, false or illegal
(Y,N or X)}
VAR     Answer:          CHAR;  {holds the answer}
        Check:           BOOLEAN;
{true when valid answer received}
        Screen:          FILE OF CHAR;
BEGIN           {yesno}
        Assign(Screen,'CON');reset(Screen);
        Check:=FALSE;
        WHILE Check=FALSE DO
        BEGIN
                Check:=TRUE;
                WRITE (Message,'?  (Y/N):');
                READ(Screen,Answer);writeln;
                IF  ( Answer=(chr(ord(13))) ) {<CR>}
                THEN CASE Default OF
                'X','x':         Check:=FALSE;
                'N','n':         Yesno:=FALSE;
                'Y','y':         Yesno:=TRUE;
                OTHERWISE
WRITELN('ERROR IN YESNO CALL (SJK)');
                END    {case Default of}
        ELSE BEGIN
IF(Answer='y')OR(Answer='Y') THEN Yesno:=TRUE
                ELSE BEGIN
IF(Answer='n')OR(Answer='N') THEN Yesno:=FALSE
                        ELSE Check:=FALSE;
                END;
           END;
        END;    {while check}
```

```
END;    {Yesno}

FUNCTION Caseup (letter: CHAR):CHAR;
{This function converts lower case letters to upper case}
{It leaves upper case and other characters unchanged}
BEGIN  {caseup}
          IF (ORD(letter)>=ORD('a')) AND
(ORD(letter)<=ORD('z')) THEN
          Caseup:=CHR(ORD(letter)-32)
          ELSE Caseup:=letter;
END;             {caseup}

{a set of procedures for modifying the screen output}
{their names should be self-explanatory              }
Procedure Unline     ;begin write(chr(1BH),'[4m');end;
Procedure Bright     ;begin write(chr(1BH),'[1m');end;
Procedure Normal     ;begin write(chr(1BH),'[0m');end;
Procedure Clearscreen;begin Write(chr(1BH),'[2J');end;
Procedure Reverse    ;begin Write(chr(1BH),'[7m');end;
Procedure Bleep      ;begin Write(chr(07H));      end;
PROCEDURE WIPE(X:integer);
{runs cursor x spaces back a line erasing as it goes !}
var y:integer;
begin
for y:=1 to X do write(chr(1BH),'[1D',' ',chr(1BH),'[1D');
end;

FUNCTION Namecheck(filename:string):boolean;
{checks that the string is an OK dos name without
extension}
{checks that is not too long so a truncated file is
opened}
begin
    If not (( ( (Pos(':',filename)=2) and (length(
filename)<=10) )
{if drive specified then 10
characters allowed in filename}
OR ( (Pos(':',filename)<>2)and (length( filename)<=8 )))
{or if drive not specified then only 8 characters allowed}
    AND (checkfn(filename))        {is legal DOS name}
    AND (Pos('.',filename)=0)  )   {has no extension}
    {and is legal DOS name}
    then
    begin
     namecheck:=false;
     writeln('bad file name - use 8 characters plus
optional drive specifier');
     writeln('do not use an extension - it is added by the
programme');
     writeln;
    end
    else namecheck:=true;
end;{namecheck}
BEGIN{  KEMPLIB  }
END. {  KEMPLIB  }
```