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GENETIC VARIATION OF AN ESTERASE SYSTEM IN SERA OF DOGS

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BRAEND, M.: *Genetic variation of an esterase system in sera of of dogs.* Acta vet. scand. 1984, *25*, 526—535. — Sera from 7 dog families comprising 20 parents with 48 offspring and 70 unrelated dogs of various breeds have been studied with regard to polymorphism of esterases. Isoelectric focusing in polyacrylamide gels of pH ranges 4.2—4.9 and 4—6 revealed great variation of phenotypes. These appeared as complex band patterns always with more than 8 zones. Treatment with an organophosphorous compound and ions of heavy metals strongly indicate that the majority of zones represent arylesterase (ArE) whereas some of the bands are cholinesterase. ArE phenotypes in families together with the appearances of band patterns in the 70 unrelated dogs are in agreement with a genetic theory of 1 locus with 5 codominant alleles. These have temporarily been named ArE^D, ArE^H, ArE^K, ArE^Q and ArE^W with ArED being the most anodal phenotype and the others in logical order. The ArE^K and ArE^Q were most common but phenotypes representing the ArE^D, ArE^H and ArE^W alleles were each observed in several animals.

serum arylesterase; isoelectric focusing; multi-band patterns; family studies; five codominant alleles.

Blood polymorphism has not been studied to such an extent in the dog as in economically important domestic animals. Thus only 13 different red cell antigenic factors have been reported (*Colling & Saison* 1978, *Saison & Colling* 1979). Polymorphisms diagnozed by the use of techniques for the separation of different types of watersoluble molecules have attracted more research interest, particularly in the last decade (for ref. see *Reetz* 1981).

At the Blood Group Laboratory, Norwegian College of Veterinary Medicine, transferrin and acidic phosphatase were shown to be polymorphic (*Braend* 1967, *Braend & Austad* 1973). During

the last year our research on blood groups of dogs has been intensified and in this paper the results from studies of an esterase system are reported.

MATERIAL AND METHODS

Serum samples from a Labrador Retriever family comprising 1 male, 7 females and 33 offspring, 6 complete families of various breeds with 15 offspring and 70 unrelated dogs were used in this study. Of the latter category, 20 were German Shepherd, 9 various types of Retriever dogs whereas the remainder came from a total of 30 breeds. The sera from the Labrador Retriever family were collected over the last 2 years and stored at —55°C when not in use.

The sera which were diluted with equal parts distilled water had glycine added to give a final concentration of 1 % and were subjected to isoelectric focusing (IEF) in 0.5 mm thick polyacrylamide gels made according to the procedures described in LKB Application Note 321 but using either Ampholine (LKB) 4—6 or Pharmalyte (Pharmacia) 4.2—4.9, instead of Immobiline (LKB). Samples were applied 2.5 cm from the cathode. Running conditions were as given in LKB Instruction 1818-P. Electrode solutions were acetic acid (0.5 mol/l) at the anode and NaOH (0.5 mol/l) at the cathode for the 4—6 pH range. For gels in the 4.2—4.9 pH range electrode solutions were L-glutamic acid (40 mmol/l) at the anode and NaOH (1 mol/l) at the cathode.

After the run the gels were incubated for 1 h at 50°C in 400 ml phosphate buffer (75 mmol/l), pH 7.5, containing 350 mg Fast Blue RR salt (Sigma F-0500) and 5 ml of 2 % α-naphtyl acetate (Sigma N-8505) dissolved in acetone. Thereafter the gels were treated as recommended in LKB Instruction 1804, but omitting the Coomassie Blue.

For identification of esterase categories the following inhibitors were added to selected samples, Neguvon (Bayer), an organophosphoric compound, giving a final concentration in the serum sample of 1 ‰, $CuSO_4$ (1 %), $MgCl_2$ (1 %), $HgCl_2$ (1 %) and EDTA (0.5 %).

RESULTS

In Fig. 1 the appearance of 13 selected serum samples are shown. The sample with the designation KK has 2 major bands,

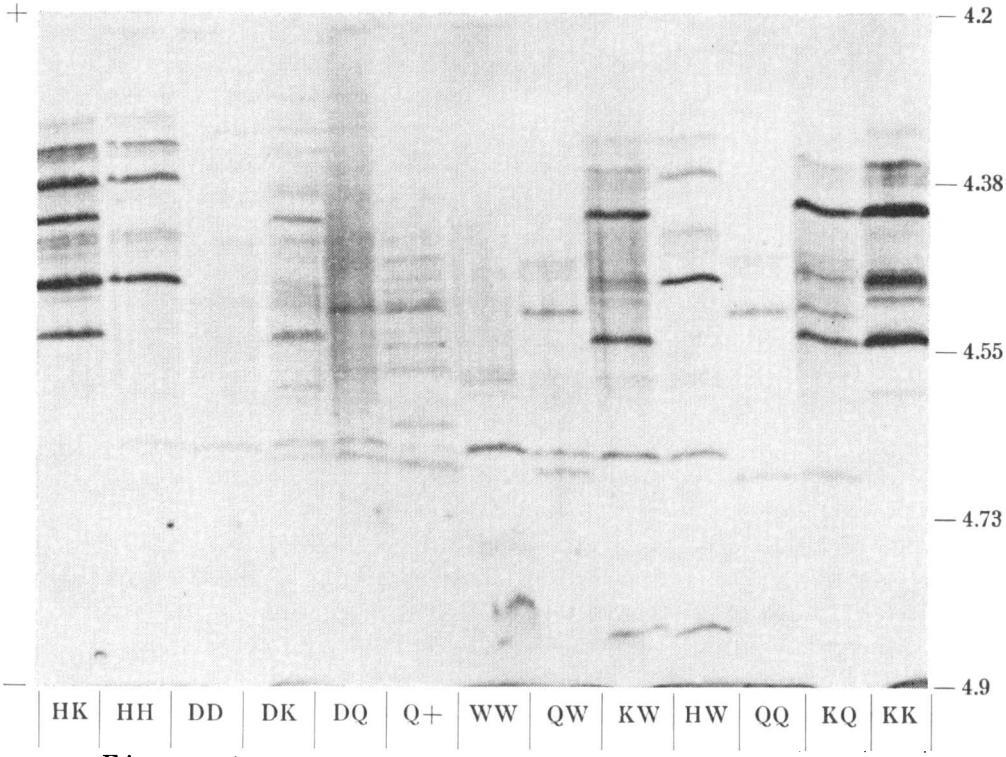

Figure 1. ArE phenotypes (genotypes) in dog sera examined in polyacrylamide gels of pH range 4.2—4.9.

one in a position corresponding to PI 4.54, the other at PI 4.41. In addition, the sample has several weak bands between the 2 major bands. Two of these bands were separate on the gel but appear as 1 thick band in the photograph. Additional weak bands are seen more anodal to the band at PI 4.41. There is also a weak band cathodal to the PI 4.54 band.

The sample designated QQ also has 2 major bands, the cathodal one at PI 4.68, the anodal one at PI 4.52. These 2 bands, however, are considerably weaker than the 2 major bands of the KK phenotype. Between the 2 major bands of the QQ pattern there are 4 weaker bands. There are also 3 weak bands anodal to the PI 4.52 band.

Between the KK and QQ phenotypes (Fig. 1) there is a sample which appears as a combination of the KK and QQ phenotype having all their bands, but weaker. This type is called KQ.

Two other phenotypes, HH and WW have similar basic band patterns as KK and QQ, but HH being more anodal and WW more cathodal. The WW, however, which has a major band at PI 4.66 with 3 weak bands in front and 3 other weak ones at PI 4.8 has a wavy band close to the cathode. This band should have been in a position of PI 4.83, as it appears in the combination phenotypes HW and KW. But it happens that bands close to the cathode do not focus properly. This is also the case with the corresponding band in the QW phenotype. Other combination phenotypes between HH, WW, KK and QQ are also shown in Fig. 1.

Three samples; DD, DK and DQ have 2 weak bands at PI 4.28—4.33. They also have several weak, more cathodal bands, the DK and DQ with the K and the Q bands in addition The sample designated Q+ has the Q pattern plus additional zones, which so far have not been named.

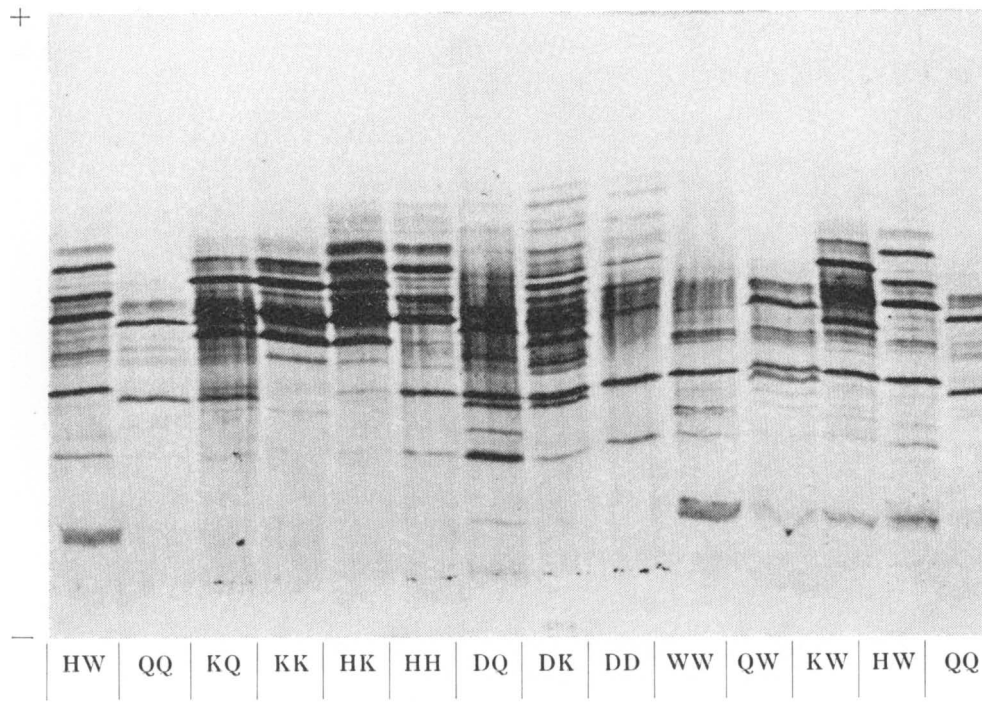

Figure 2. ArE phenotypes in dog sera examined in polyacrylamide gels of pH range 4—6.

Fig. 2 is a photograph of a gel with pH range 4—6 in which 12 selected samples have been run, 2 of them being repeated. The zones appear sharper and more distinct than in gels of pH range 4.2—4.9, but since bands are less separated at the pH 4—6 range correct diagnosis of phenotypes cannot always be done. The HH phenotype is an example. This type has 2 weak bands in the position of the main D bands, but in gels of pH range 4.2—4.9 these 2 bands are clearly different in position from the 2 D bands.

In Fig. 3 the effects of Neguvon is shown. The bands marked with arrows were affected by Neguvon, but not the other esterase bands. $CuSO_4$ also gave a similar effect. EDTA and $MgCl_2$, however, did not inhibit the expression of any bands. $HgCl_2$ on the other hand inhibited the expression of all esterase bands.

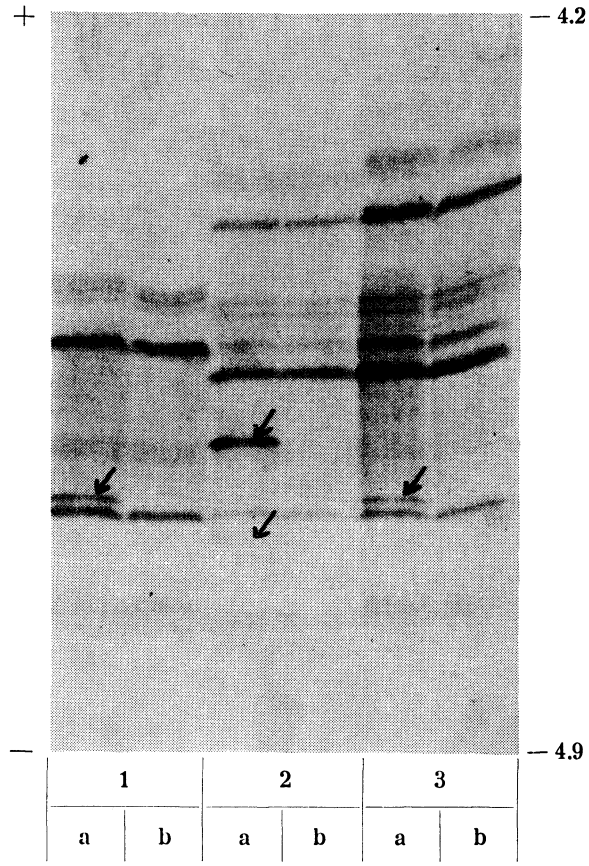

Figure 3. An ArE QQ phenotype (no. 1) and two KQ phenotypes (nos. 2 and 3), untreated (a) and treated with Neguvon (b).

On the basis of these results, it is concluded that the esterase bands of the system unaffected by Neguvon and $CuSO_4$ is arylesterase (*Augustinsson* 1961) whereas those zones which were affected may represent cholinesterase.

The appearance of the band patterns described above suggests a genetic theory of 1 locus with 5 codominant alleles. These have temporarily been named; ArE^D, ArE^H, ArE^K, ArE^Q and ArE^W. In the Labrador Retriever family (Table 1), the ArE phenotypes were in agreement with the genetic theory. They had, however, weakened during the 11 years storage.

Table 1. ArE phenotypes in the family of a male Labrador Retriever dog, type KQ.

Female no.	Type	Types of offspring (nos. in parentheses)		
1	KQ		KQ (3)	QQ (1)
2	QQ		KQ (2)	QQ (3)
3	QQ		KQ (2)	QQ (1)
4	KQ	KK (2)	KQ (4)	
5	QQ		KQ (5)	QQ (1)
6	QQ			QQ (2)
7	QQ		KQ (4)	QQ (3)

The ArE phenotypes in the 6 families with altogether 15 offspring were also in agreement with the genetic theory. In 1 family the female gave the ArE^W allele to 2 offspring. In 2 other families 2 offspring got the ArE^H allele either from the dam or the sire. The remaining had phenotypes controlled by the ArE^K and ArE^Q alleles.

For the fifth allele, ArE^D, no family data were available, but among the 70 dogs of various breeds the D pattern appeared in two Vorsteher, one being DK, the other DQ. It was observed in 5 German Shepherd dogs, 2 being DD, 2 DK and 1 DQ. In the remainder of the 70 dogs the phenotypes controlled by the ArE^K and ArE^Q alleles were most common. The allele ArE^W was found in a Hamilton hound and an Afghan dog, both QW, a Pointer, HW and a Curly Coated Retriever, WW. The ArE^H allele occurred in an Elkhound and a West Highland White Terrier, both HQ, a Pointer, HW and a Bearded Collie, HH.

Based on the family data and the appearance of the band patterns it is concluded that the genetic theory is correct. Of the

15 possible alleles 13 have been found. The missing phenotypes are DH and DW. The one exception to the genetic theory (Q+ in Fig. 1) is assumed to be a heterozygote between ArE^{Q} and an allele so far not seen in any other animal.

DISCUSSION

Augustinsson (1961) who did extensive comparative studies on various forms of esterase present in vertebrate plasma revealed that 3 groups of esterase existed in his material: arylesterase (ArE), aliesterase (AliE) and cholinesterase (ChE). According to him and his associates arylesterase and cholinesterase are present in dog plasma with arylesterase expressing the highest relative activity. They further reported that in most species arylesterase is inhibited by EDTA and cations of heavy metals but not by organophosphorous compounds. In the present studies EDTA and Mg ions did not inhibit the expression of esterase zones, whereas Hg ions did. The organophosphorous compound Neguvon inhibited certain esterase bands, but not the majority of these. Cu ions also had some effect on the Neguvon sensitive bands but not as clearcut. This evidence indicates that the Neguvon sensitive bands in dogs are cholinesterase. Consequently, the observed band patterns represent 2 overlapping systems, the zones of the ArE system being the dominating and most complex ones with the technique employed. The difference in sensitivity to Neguvon proved useful in establishing the ArE gene controlled band patterns since prior to that finding certain phenotypes did not fit with the genetic theory. In discussing the effect of inhibitors it further has to be mentioned that in samples from heparinized bloods the zones were usually more anodal in the gel, being curvy and not well focused.

Simonsen (1976) assumed that esterase of dogs is polymorphic but did not undertake any further study. *Sugiura et al.* (1977) used starch gel electrophoresis and reported polymorphism of an eserine resistant esterase. They observed 6 phenotypes in very large sample material, their results being in agreement with a genetic theory of 3 codominant alleles. They did not, however, attempt any further classification relative to *Augustinsson*'s 3 groups of esterase, except for concluding that the system which they studied was not cholinesterase. Thus they did not state that their eserine resistant esterase was arylesterase.

Sugiura et al. (1977) used the AB nomenclature. In the present study the genes and phenotypes are temporarily named according to principles outlined by *Braend* (1965). By utilizing the whole alphabet already in the original report on a polymorphic system it will be possible to give logical symbols to additional alleles and phenotypes in agreement with their migration rates or isoelectric point. When naming the most anodal phenotype ArED and the most cathodal one for ArEW, the remaining letters are left free to give logical symbols to additional phenotypes. And new alleles will most probably be found since as many as 5 have been recognized in a study which is rather limited with regard to number of dogs.

The gene product of the ArE system as exemplified by the phenotypic band patterns comprises a large number of bands of which 2 are relatively heavy and the remaining ones weak. The positions of bands were given in Results with figures extrapolated from the pH range 4.2—4.9. The figures for the PI'es are, however, only approximate. They are given merely for the description of band patterns. The exact PI of individual bands may be different from those given in connection with Fig. 1, because the gradient may not be strictly linear and also because the position of bands depends on running conditions, such as time. It is obvious, however, that this is a system of very complex gene products. The reason for this complexity can at this stage only be a matter for speculation and shall therefore, not be discussed.

A significant finding in the present study is the differences in expression of ArE zones. This was particularly the case for gels of 4.2—4.9, but could also be seen in gels of pH range 4—6. The reasons for the variability in expression of bands could be several. It could be genetically controlled such as in pigs. The plasma activity of arylesterase in this species is under the control of at least 8 alleles (*Augustinsson & Olsson* 1960, *Gahne et al.* 1972). The alleles (with the technique of *Gahne et al.* 1972) determine activity of 0, 120, 240, 300, 360, 420, 480 and 600 units respectively in Swedish Landrace pigs. Another explanation for the variable expression of bands might be a combined action of genes and environment. Sex hormones may also influence the activity of esterase. *Augustinsson & Olsson* (1960) found that testosteron in boars influenced the activity of arylesterase, the esterase activity being higher before sexual maturity and in castrated boars than in sexually mature boars. The material in

the present study was not suitable or comprehensive enough for a study of which factors are of importance for the variability in expression of bands. Selected animals and larger sample material kept under controlled environmental conditions would be necessary for such a study to be properly undertaken.

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SAMMENDRAG

Genetisk variasjon av et esterase system i sera fra hund.

Esterase polymorfisme er blitt undersøkt i serum prøver fra 7 hundefamilier, 20 foreldre med 48 avkom, og i 70 prøver fra ubeslektede hunder av forskjellige raser. Isoelektrisk fokusering i polyacrylamidgeler i pH områdene 4.2—4.9 og 4—6 og med spesifikk farging resulterte i et stort antall forskjellige fenotyper. Disse viste seg som komplekse båndmønstre, alltid med flere enn 8 bånd. Behandling med en organisk fosforforbindelse og med joner av tunge metaller tyder på at flesteparten av disse bånd er arylesterase (ArE) mens noen av dem er cholinesterase. ArE fenotypene i det undersøkte materiale tyder på en genetisk teori med et locus og fem kodominante alleler. Disse har fått den foreløpige betegnelse ArE^D, ArE^H, ArE^K, ArE^Q og ArE^W hvor ArED fenotypen har sin plass nærmest anoden og med de andre i logisk orden. ArE^K og ArE^Q allelene var vanligst, men fenotyper av ArE^D, ArE^H og ArE^W ble alle observert i flere dyr.

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