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Variation of Transferrin and Esterase in Sera of Dogs

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Braend, M. and A. E. Andersen: Variation of transferrin and esterase in sera of dogs. Acta vet. scand. 1987, 28, 435–444. – Transferrin (Tf), arylesterase (ArE) and another esterase (Es) have been studied in sera from 1023 dogs by the use of isoelectric focusing (IEF) in polyacrylamide gels. Tf types were determined after protein staining in gels of pH range 5-6 and 5-7. The expression of Tf types as measured by strength of bands varied considerably. The Tf band patterns are explained by the occurrence of the 4 codominant alleles, Tf^F, Tf^{M_1}, Tf^{M_2} and Tf^S of which Tf^{M_1} and Tf^{M_2} are common. Some breeds had similar gene frequencies, others differed considerably. For determination of ArE types specifically stained gels of pH range 4.2-4.9 and 4.0-6.5 were employed. The ArE phenotypes appeared as multiple band patterns of which the individual bands varied considerably in strength. Atypical ArE patterns were observed in dogs suffering from certain diseases. The normal ArE phenotypes are explained by a total of 7 codominant alleles of which ArE^N and ArE^T have not been previously described. Gene frequencies varied between breeds. For the other esterase (Es) the appearance and position of bands indicate at least 2 alleles in this system.

canine; isoelectric focusing; expression; polymorphism; genes; frequencies; breeds; comparisons.

Introduction

Blood polymorphisms have not been studied to the same extent in dogs as in other domestic animals. But a number of investigations on transferrin has been undertaken during the last 2 decades (for ref. see *Reetz* 1981 and *Juneja et al.* 1981) and a total of 5 alleles has been reported. With regard to esterase *Sugiura et al.* (1977) reported 6 eserine resistant types explained by 3 alleles, whereas *Braend* (1984) using IEF observed multiple band patterns which could be explained by 5 codominant alleles.

For solving canine parentage problems it was found necessary to improve the knowledge of useful serum protein systems and the distribution of types between breeds. In the present report results from investigations on Tf, ArE and Es systems are given.

Material and methods

Serum samples were obtained from 850 dogs which were patients at Department of Internal Medicine II, Norwegian College of Veterinary Medicine or Stovner Animal Clinic, Oslo. Samples sent to the Blood Group Laboratory, Department of Internal Medicine I for the purpose of solving parentage problems have also been utilized in this study. They total 25 bitches, 104 offspring and 44 real or suspected fathers. The samples were collected over the last 4 years and stored at -55°C when not in use.

The sera came from a total of 96 breeds. For 6 breeds the number of dogs examined exceeded 28, 12 breeds had from 10 to 22 and the remaining breeds were represented with 1 to 9 animals.

The technique for determining esterase types

was the same as described by *Braend* (1984), using α-naphtyl acetate as substrate and Fast Blue RR salt as the dye. For determination of the transferrin type polyacrylamide gels were made in the same way as for the esterase gels but using Pharmalyte of pH range 5-6 or Ampholine 5-7, with running time 2½ h at maximum 2000 V, 25 mA and 25 W (as for the esterase gels). The Tf gels were stained with Coomassie Blue according to procedure given in LKB Application Note 321.

The iron binding capacity of the Tf bands was tested by the use of ironcitrate-^{59}Fe (spec. activity 0.4 M Bq/μg Fe). To 125 μl serum and 125 μl distilled water, 25 μl 10% glycine and 50 μl ^{59}Fe citrate with a total activity of 21,000 or 33,000 Bq were added. After IEF the gels were wrapped in cling film and placed on X-ray film for 4 or 6 days. Development was according to the standard method for X-ray films. To a few selected samples the organophosphoric compound Neguvon (Bayer) which is a cholinesterase inhibitor, was added, giving a final concentration of 2‰ in the mixture.

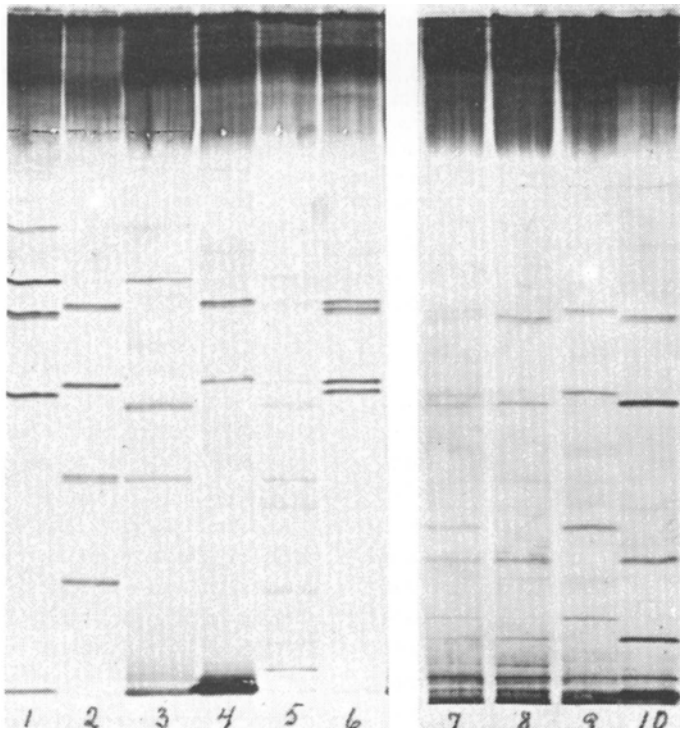

Figure 1. Photograph of two portions of polyacrylamide gels, pH range 5-6, after IEF, showing selected Tf phenotypes, the number of Tf bands in parantheses.
No 1: FM_2 (4), No 2: M_1S (4), No 3: FF (4),
No 4: M_1M_1 (2), No 5: FM_1 (8) weak, No 6: M_1M_2 (4),
No 7: M_1M_2 (8) weak, No 8: M_2M_2 (4), No 9: M_1M_1 (4),
No 10: M_2M_2 (4).

Results

Fig. 1 is a photograph of 2 portions of polyacrylamide gels, pH range 5-6. The M_1 type appears always with 2 distinct Tf bands, namely the 2 strong bands shown in sample no 4. In addition, as shown in samples 7 & 9, the M_1 type may have 2 other, more cathodal bands. These latter bands have also the ability to bind ^{59}Fe. This is shown in Fig. 2 which is a photograph of an autoradiograph. But they do not always appear, as in samples 4 & 6 (Fig. 1). In gels of pH range 5-7 they cannot be diagnozed with certainty after protein staining but they may bind ^{59}Fe. The position of the M_2 bands is shown in samples 1, 6, 7, 8 & 10. The M_2 type may also have 2 bands in a more cathodal position. They do not, however, appear in samples 1 & 6 whereas they are shown in samples 7, 8 & 10 (Fig. 1). Sample no 1 has 2 bands in a more anodal position. These represent the F type. The F type may also have 2 more cathodal bands. They are shown in sample no 3. The F type is also seen in samples no 5, although weak. A fourth 2 band pattern is shown in sample no 2. This is the S type.

Inheritance of Tf types was examined in 25 parentage cases. All offspring had Tf patterns compatible with those of their dams. The compatibility with the putative fathers had to vary. But in cases with the involvement of 2 male dogs and where 1 of the suspected fathers could be excluded by the use of other systems, the other father was found to fit.

Frequencies of Tf types are shown in Table 1. The great majority of dogs have the M_1, M_2 or M_1 M_2 type. Among the 6 common breeds only German Shepherd had the F type. But F was found in Dobermann Pincher with 4 of 21, in German Wirehaired Pointer with 5 of 15 and in Poodle with 3 of 14. The 6 breeds in Table 1 were all in genetic equilibrium, since the observed and expected frequencies show close agreement. The pooled material comprising 90 breeds was not tested for genetic equilibrium. Golden Retriever, Boxer and English Setter had virtually identical figures of $Tf^{M}{}_1$ and $Tf^{M}{}_2$ alleles, whereas the 3 other breeds in Table 1 had significant differences between allele frequencies. The Tf^{S} allele was found in 2 of 8 Husky and 1 of 10 Elkhound. It also occurred in 1 Breton and 1 Berner Sennen. In addition, in a Husky parentage case there were 2 homozygous and 2 heterozygous S. In an Elkhound control case there were 2 heterozygous of 3.

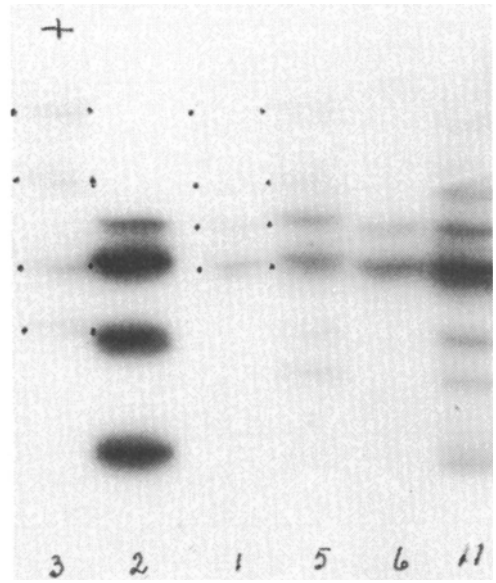

Figure 2. Photograph of an autoradiograph of a polyacrylamide gel, pH range 5-7, after IEF, showing five of the samples in Fig. 1.
No 1: FM_2, No 2: M_1S, No 3: FF, No 5: FM_1, No 6: M_1M_2.
Sample No 11 is an additional FM_1.
Weak bands in samples 1 & 3 are marked with dots. Notice the difference in strength of bands.
Each sample had ^{59}Fe with 21000 Bq.

Table 1. Transferrin types of dogs from 6 common breeds and in the pooled population material of 90 breeds.

			Phenotypes						Genes		
	n		FF	FM_1	FM_2	M_1M_1	M_1M_2	M_2M_2	Tf^F	Tf^M	Tf^{M_2}
German	148	obs[3]		3	4	47	68	26	.02	.56	.42
Shepherd		exp[4]	0.06	3.32	2.49	46.41	69.62	26.11			
Labrador	74	obs				29	40	5		.66	.34
Retriever		exp				32.23	33.21	8.55			
Golden	68	obs				13	28	27		.40	.60
Retriever		exp				10.88	32.64	24.48			
Boxer	35	obs				4	20	11		.40	.60
		exp				5.60	16.80	12.60			
English	32	obs				5	18	9		.44	.56
Setter		exp				6.20	15.77	10.03			
Rott-	28	obs				2	8	18		.21	.79
weiler		exp				1.23	9.29	17.47			
			Observed phenotypes								
Ninety	455	FM_1	FM_2	M_1M_1	M_1M_2	M_2M_2	M_2S		.01	.47	.52
breeds		7	5	117	183	138	5		Tf^S =	.005	

1) Only the population material not the parentage material was utilized for frequency estimates
2) Genes were determined by counting from the phenotypes
3) Obs = observed
4) Exp = expected under genetic equilibrium

In Fig. 3 esterase types are shown. The ArEN pattern occurs in samples 8, 9 and 10, as an assumed homozygote and 2 heterozygotes. The other samples in Fig. 3 show various other ArE phenotypes. As reported by *Braend* (1984) it can also be seen that there are minor bands, more or less distinct though, between the 2 major bands and also more anodal to these. The D pattern, however, is special. It has 2 very sharp bands with a weaker 1 more anodal and several weak ones in a more cathodal position. Sample no 5 has bands additional to the D pattern. These latter ones may be controlled by an additional allele.

Types which are assumed to be another esterase (Es) are shown in all samples in Fig. 3. Sample no 2 has a 2 band pattern called C. All the other samples have a 2 band pattern designated G, but the 2 bands are very weak in samples 3 and 7. Since the Es bands often are weak no systematic genetic studies have been undertaken.

In Fig. 4 three additional ArE phenotypes are shown, without and with Neguvon treatment. The NQ sample has been included for comparison. The 2 other samples have two Neguvon resistant zones in a position different from any of the previously described ArE patterns. This new type is called T. It was always weak and only occurred in 4 German Shorthaired Pointer, where it also was found in combination with Q.

Fig. 5 shows 2 atypical phenotypes. They appear with a large number of bands which do not fit with the regular band patterns. The sample in the middle came from a dog which had a diagnosis of hepatopathia and by autopsy found to have pancreas-adenocarcinoma. The other dog with atypical pattern in Fig. 5 had diabetes mellitus and adenocarcinoma in the lungs. Two other dogs also had

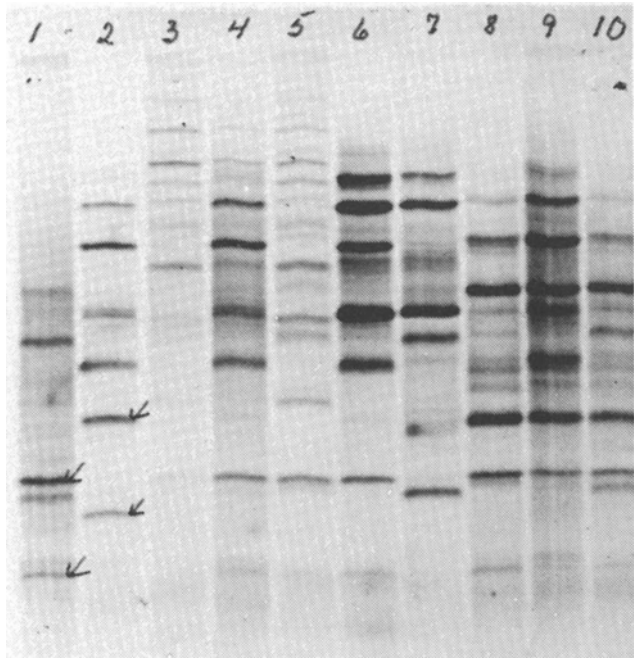

Figure 3. Photograph of portion of a stained polyacrylamide gel, pH range 4.2-4.9, after IEF, showing selected ArE phenotypes.
No 1: QQ, No 2: KK, No 3: D, No 4: DK, No 5: D^+,
No 6: HK, No 7: HQ, No 8: NN, No 9: KN, No 10: NQ.
Esterase bands in samples 1 & 2 are marked with arrows, No 1 being a G, No 2 a C.

atypical ArE patterns. One suffered from purulent metritis. The other had hemolytic anemia and icterus. They both had strong Neguvon resistant zones close to the cathode. One of those 2 dogs had very weak Q zones, the ArE type of the other could not be determined. Three dogs did not show any ArE zones, but they had Es zones. One of these 3 suffered from general infection and purulent arthritis. It was retyped after a year. It then had recovered and showed a weak Q. The second dog who had a diagnosis of pancreas insufficiency was retyped after 2 years. It then had a very weak W. The third dog who also suffered from pancreas insufficiency had died. In addition to the 7 dogs mentioned above there were 3 others which could not be diagnozed as to ArE types.

Inheritance of ArE types in the families of the 25 bitches was according to theory. The offspring all had types being compatible with those of the respective bitches. Thus, 1 bitch with D had 2 of 5 offspring also with D. Both putative fathers had Q. In 4 families the H type segregated according to theory. For the new types N and T there was no family material but the appearance of their

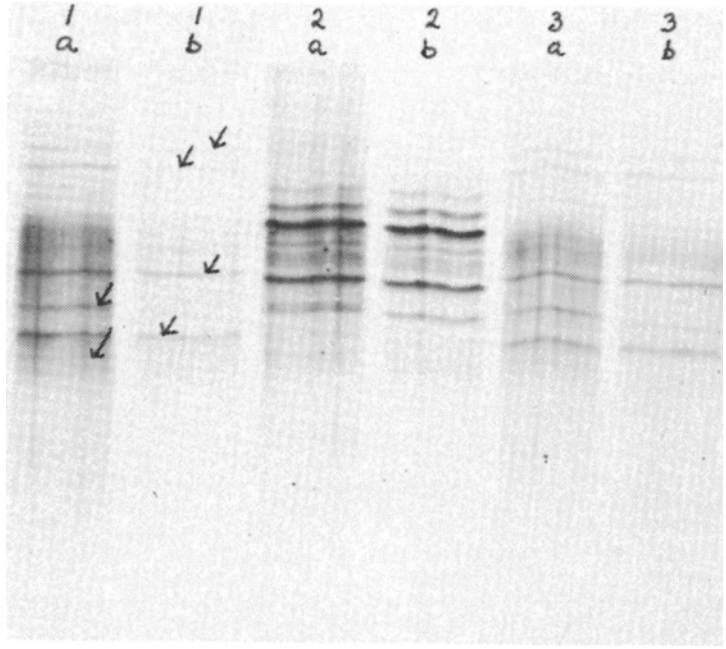

Figure 4. Photograph of portion of a polyacrylamide gel, pH range 4.0-6.5, after IEF, showing the effect of Neguvon on three selected samples. The sample marked with a is untreated, the one marked with b is Neguvon-treated.
No 1: DT, No 2: NQ, No 3: DT.
The Es bands of la and the major ArE bands of 1b are marked with arrows.

band patterns and their Neguvon resistance strongly suggest that they are allele products additional to those 5 described earlier.

Observed and expected frequencies of ArE phenotypes and their genes are shown in Table 2 and 3. The 6 breeds showed genetic equilibrium except for German Shepherd where there was a surplus of homozygotes to such an extent that it was close to reaching significance at the 5% level. It should further be noted that frequencies of the Tf^N support the theory of it being an additional allele. Among the 90 breeds the D type occurred in 7 of 16 German Shorthaired Pointer and 4 of 15 German Wirehaired Pointer. It was also found in Dobermann Pincher and Pointer, with 2 of 21 and 2 of 11 respectively. The H type was observed in 5 of 11 Pointer, 4 of 8 Husky and 3 of 11 Elkhound, parentage material not included. But it was also observed in a number of other breeds. The N type was besides in Rottweiler found in 3 out of 6 Leonberger. The W type occurred in many breeds and relatively frequent in some. Of 3 Shi Tsu 1 was homozygous W, the other 2 heterozygous.

Discussion

The technique used in this study usually offered no problem in determining the Tf phe-

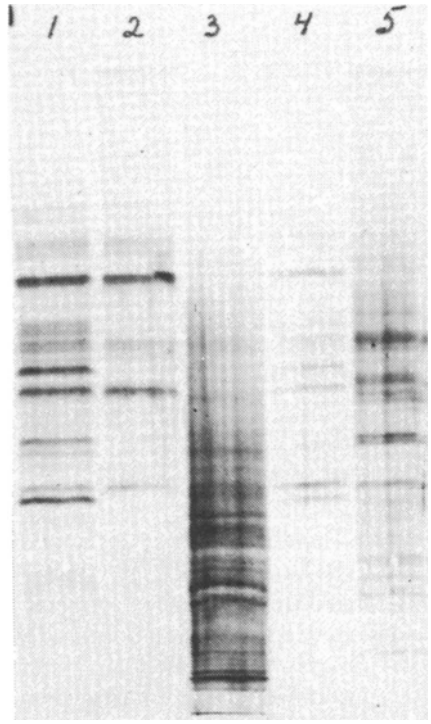

Figure 5. Photograph of portion of a polyacrylamide gel, pH range 4.2-4.9, after IEF, showing selected ArE phenotypes.
No 1: KQ, No 2: KK, No 3: atypical, No 4: KQ,
No 5: atypical.

notypes except for rare cases where the bands appeared very weak. However, in such cases the band strength could be improved by increasing the amount of serum applied on the gel. The reasons for the observed differences in band strength can at this stage only be a matter of speculation and shall therefore, not be elaborated on. But it should be mentioned that it might be genetically or environmentally conditioned, or by a combination of these. In this connection the genetic control of the band patterns as such is relevant. The gene product appears as 4 distinct bands of which the cathodal ones might be very weak or not seen at all. The 4 band pattern is in contrast to what has been previously reported. Thus when Tf polymorphism in dogs was first described by *Braend* (1967) the basic band pattern appeared as 3 bands on autoradiographs. *Stevens & Townsley* (1970) and *Juneja et al.* (1981) also reported a 3 band pattern as being the gene product. The reason for this discrepancy is most probably due to the technique. However, a change from 3 to 4 is not easy to explain. It might be that after IEF 6 bands should appear but that 2 of them are so weak that they are not observed. Thus there were gels in which an additional anodal band was indicated. It may be that a similar situation

Table 2. ArE types in the 3 most common breeds and in the pooled population material of 90 breeds.

			Phenotypes										Genes			
	n		DD	DK	DQ	DW	KK	KQ	KW	QQ	QW	WW	ArE^D	ArE^K	ArE^Q	ArE^W
German	148	obs	2	3	5		61	49	3	24	1		.04	.60	.35	.01
Shepherd		exp	0.24	7.10	4.14	0.12	53.28	62.16	1.78	18.13	1.01	0.01				
Labrador	74	obs					8	31		35				.32	.68	
Retriever		exp					7.58	32.20		34.22						
Golden	68	obs					4	13	2	43	6			.17	.77	.06
Retriever		exp					1.97	17.80	1.39	40.32	6.28	0.24				

		Observed phenotypes														
Ninety	455	DD	DK	DQ	DT	HH	HK	HQ	HW	KK	KQ	KW	NN	NQ	QQ	QT	QW	WW
breeds		1	8	8	2	6	10	13	4	103	122	19	1	2	125	2	21	8

Genes

$ArE^D = .02$, $ArE^H = .04$, $ArE^K = .40$, $ArE^N = .004$, $ArE^Q = .46$, $ArE^T = .004$, $ArE^W = .07$

Table 3. ArE types in 3 other common dog breeds.

	n		Phenotypes									Genes			
			HH	HK	HQ	KK	KN	KQ	NN	NQ	QQ	ArE^H	ArE^K	ArE^N	ArE^Q
Boxer	35	obs				27		7			1		.87		.13
		exp				26.49		7.92			0.59				
English	32	obs			2	9		16			5	.03	.53		.44
Setter		exp	0.03	1.02	0.84	8.99		14.92			6.20				
Rott-	28	obs				5	4	10	1	2	6		.43	.14	.43
weiler		exp				5.18	3.37	10.36	0.55	3.37	5.18				

exists for dogs as in cattle where the Tf gene product consists of 4 band. *Maeda et al.* (1984) reported the difference between the 4 bands to be due to a combination of differences in sialic acid and a scission between amino acid 624 and 625 in the Tf chain which they assumed to be 678 amino acids long as in man.

The nomenclature used for the 4 alleles found in the present study is based on that employed by *Braend* (1967) and *Reetz* (1981). *Bernoco et al.* (1966) also used the same nomenclature as *Braend* whereas *Stevens & Townsley* (1970), *Vriesendorp et al* (1973) and *Juneja et al.* (1981) used the AB nomenclature. A fifth allele has been reported by *Vriesendorp et al.* (1973), *Clark et al.* (1975) and *Juneja et al.* (1981), by the latter called D. In the present study a fifth allele was indicated in a dog of unknown breed. This might be the same as Tf^D. Otherwise the Tf^{M_1} and Tf^{M_2} alleles were most common. This agrees with the studies of *Reetz* (1981) and *Juneja et al.* (1981). With regard to comparisons between breeds the numbers of dogs investigated often are rather small. But for some breeds a sufficient high number has been investigated. This applies to German Shepherd which appeared with very similar Tf frequencies in the present study as in that of *Juneja et al.* (1981).

The band patterns of the esterase phenotypes varied even more in strength of individual bands than did the transferrins. There may be various explanations for these differences. Thus sex hormones may be of influence (*Augustinsson & Olsson* 1960), but the environment and genetics could also play a role. The material was not appropriate for exploring all the various possibilities. But our results should offer some explanations.

Three of the dogs did not show any ArE bands. One of them who suffered from general infection recovered and appeared with weak ArE zones a year later. The reason for the disappearance of ArE zones at the first testing might have been due to the infection which for some reason depleted the ArE content in serum. Another of the negative dogs which suffered from pancreas insufficiency, and which is on constant treatment with Pankreon comp forte (Meda, Copenhagen), showed very weak W bands 2 years later. If pancreas is an important organ for the production of arylesterase a malfunction might explain the disappearance of ArE zones. On the other hand there were 14 German Shepherd and 1 Collie in the study which also had a diagnosis of pancreas insufficiency, but of which only 4 showed weak bands whereas the others had normal band strength. Interesting though is the fact that the Es bands were not affected in the 3 dogs mentioned above. Other atypical ArE patterns had so many bands and in such positions that their phenotype could not be determined (Fig. 5).

Braend (1984) based his conclusions and nomenclature of dog esterases on the original work of Augustinsson (1961) who after extensive studies reported 2 esterases in plasma of dogs, arylesterase and cholinesterase. Accordingly, Braend (1984) assumed that the two systems which be observed were arylesterase and cholinesterase, since one of the systems was inhibited by Neguvon. Both arylesterase and carboxylesterase show high concentrations in rat liver (Knox 1972, Dixon & Webb 1979). In pancreas of rats, however, there is no arylesterase, but the amount of carboxylesterase is 5 times higher than in the liver. If canine ArE has pancreas as one of its production sites it could explain the diseappearance of the ArE bands in the dog with pancreas insuffiency. On the other hand, it would be in contrast to the situation in rats. For the other esterase system in dogs (Braend unpublished) it appears that it is resistant to eserine whereas cholinesterase is inhibited by eserine (Augustinsson 1961). Consequently, the inhibition pattern of the second system does neither fit with arylesterase nor with cholinesterase. Since carboxylesterase also is inhibited by organophosphorous compounds the second system may be carboxylesterase (Es). As judged by number and strength of bands, however, it is a very minor one compared to the ArE system, which may explain the findings of Augustinsson (1961).

Another possible explanation for lack of ArE zones could be the occurrence of a null allele, which is reported for aliesterase in horses (*Gahne* 1966) and arylesterase in pigs (*Augustinsson & Olsson* 1960). In the present investigation a null allele must be excluded for the 2 dogs mentioned above. The third dog, also a German Shepherd, could unfortunately not be retyped. However, the oberved ArE frequencies in German Shepherd might indicate the occurrence of a null allele since there is a surplus of homozygotes. On the other hand, many of the dogs in the present study suffered from diseases and might have had some of its bands too weak to be diagnozed. For the time being and because of lack of proper family data the question of the occurrence of a null allele therefore, cannot be answered.

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Sammendrag

Variasjon av transferrin og esterase i hundesera.

Transferrin (Tf), arylesterase (ArE) og en annen esterase (Es) er ved bruk av isoelektrisk fokusering (IEF) i polyakrylamid geler undersøkt i sera fra 1023 hunder. Tf typene ble bestemt i geler med pH områder 5-6 og 5-7 etter protein farging. Typenes uttrykksform, bedømt etter båndmønstre, viste stor variasjon. Båndmønstrene forklares med forekomst av de 4 kodominante alleler, Tf^F, Tf^{M_1}, Tf^{M_2} og Tf^S hvorav Tf^{M_1} og Tf^{M_2} er vanlige. Genfrekvenser var de samme for visse raser, men varierte for andre. For diagnostisering av ArE typer ble det brukt spesifikk farging av geler med pH områder 4.2-4.9 og 4.0-6.5. ArE fenotypene opptrådte som multiple båndmønstre hvor de individuelle bånd viste stor variasjon m.h.t. styrke. Atypiske ArE mønstre ble observert i hunder med visse sjukdommer. De normale ArE fenotyper forklares gjennom forekomst av 7 kodominante alleler hvorav ArE^N og ArE^T ikke tidligere er beskrevet. Det var variasjon av genfrekvenser mellom raser. For den andre esterase (Es) tyder utseende og plassering av bånd på minst 2 alleler i dette system.

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