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# Electrophoretic studies on the blood proteins of domestic dogs and other Canidae

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This report gives a review of electrophoretic studies on dogs and describes the zymograms of 18 enzymes and 3 proteins in blood from dogs and their relatives. 14 of the enzymes and one protein have identical zymograms in dogs, representing 10 different breeds. The same 14 enzymes and the protein are electrophoretically identical in the wolf and the dog. The jackal and the dog differ in one case, the Hallstrom-dog and the domestic dog in two cases, the coyote and the dog in three cases, and the red fox and the dog in six cases.

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There has been a number of previous studies of electrophoretic variation in domestic dogs and related Canidae. This paper reviews these studies and presents new data, with three objectives:

(1) To estimate the level of electrophoretic variation in domestic dogs.
(2) To look for effects at the protein level of the selection to which dogs have been subjected by man.
(3) To see whether isoenzymes can be used to clarify the taxonomic relationships between domestic dogs and their relatives.

Most previous workers searching for electrophoretic variation in dogs and other Canidae have looked at only one or two proteins each, and their results are summarized in Tables 1 and 2. Two recent independent investigations describe the zymograms of many proteins, some of those overlapping the present work. These two studies and the present work are summarized in Table 8. MEERA KHAN et al. (1973) used mongrels for the work, and WEIDEN et al. (1974) used Labradors, Basenji and mongrels of those two breeds, both studies demonstrating a rather small electrophoretic variation in domestic dogs.

The dog is one of the oldest domesticated animals. Studies of the C-14 content of dog skeletons found with human remains suggest that dogs have been associated with man for 7—12,000 years (DEGERBØL 1961, 1961—62; LAWRENCE 1966; MCMILLAN 1970). During this time conscious and unconscious selection by man has moulded dogs into a bewildering variety of breeds, showing a wide range of morphological characteristics. It is of interest to see whether this selection for visible traits has been accompanied by selection on the invisible variation shown by blood proteins. Protein differences might be found between breeds, or between groups of breeds on different evolutionary branches. The different branches of dog evolution have been traced using morphological characters and the breeds are divided into four groups (MOORE 1962; FIENNES and FIENNES 1968), into five groups (STUDER 1901; ZEUNER 1963) or subdivided into eight groups (ANTONIUS 1922). Neither MEERA KHAN et al. (1973) nor WEIDEN et al. (1974) have correlated their results with the groups of breeds, and the only indication of inter-group differences, found by simple electrophoresis, comes from the work of TANABE et al. (1974) on leucine aminopeptidase. TANABE et al. (1974) used a combination of STUDER's and ANTONIUS's groupings, so that they recognise five groups and an extra one for Japanese native dogs. They found a significant difference in the gene

*Table 1.* Previous electrophoretic work in dogs

| Protein | Source | Results | Determined by | References |
|---|---|---|---|---|
| Acid phosphatase (Ap) | Red blood cells | Polymorphic | 1 autosomal locus with 2 codominant alleles | BRÆND and AUSTAD 1973 |
| Albumin (Alb) | Serum | Polymorphic | 1 autosomal locus with 2 codominant alleles | DAY et al. 1971 |
| Amylase (AMY) | Serum | Two zones with activity | — | SEARCY et al. 1966 |
| Carbonic anhydrase (CA) | Red blood cells | 1 band | — | BYVOET and GOTTI 1967<br>FUNAKOSI and DEUTSCH 1971 |
| Catalase (CAT) | Red blood cells | Polymorphic | 1 autosomal locus with 2 alleles | ALLISON et al. 1957<br>FERNSTEIN et al. 1968<br>WONG et al. 1972 |
| Esterase (P-EST) | Plasma | An arylesterase and a cholinesterase | — | AUGUSTINSSON 1961 |
| Glucose-6-phosphate dehydrogenase (G-6-PD) | Red blood cells | 1 band | — | NAIK et al. 1971<br>KAMADA and HORI 1970 |
| Glutamic-oxaloacetic-transaminase (Got) | Heart | 2 fractions | — | FLEISHER et al. 1960 |
| Haptoglobin (Hp) | Serum | Polymorphic | — | NAIK et al. 1971 |
| Hemoglobin (Hb) | Red blood cells | 1 band | — | NAIK et al. 1971 |
| Leucine aminopeptidase (Lap) | Plasma | Polymorphic | 1 autosomal locus with 2 codominant alleles | TANABE et al. 1974 |
| 6-phosphogluconate-dehydrogenase (6-PGD) | Red blood cells | 1 heavy band, 1 weak band | — | NAIK et al. 1971 |
| Pepsinogen | Gastric juice and urine | Sometimes present, sometimes absent | — | HANLEY et al. 1966 |
| Peptidase D (Pep D) | Red blood cells | Polymorphic | 1 autosomal locus with 2 codominant alleles | SAISON 1972 |
| Tetrazolium oxidase (To) | Red blood cells | Polymorphic | 1 autosomal locus with 2 codominant alleles | BAUR and SCHORR 1969 |
| Transferrins (Tf) | Serum | Polymorphic | 1 autosomal locus with 3 codominant alleles | BRÆND 1966, STEVENS and TOWNSLEY 1970 |

frequency of leucine aminopeptidase, two of the groups were fixed for $Lap^A$, two had 0.17 in gene frequency of $Lap^B$, one 0.04, and one 0.02. Their work indicates that it is possible to find some correlations between the grouping based on isoenzyme studies and the grouping based on morphological studies.

Although it is generally agreed that dogs are descended from wolves (STUDER 1901; DEGERBØL 1961; BAUME 1962; LAWRENCE 1966; FIENNES and FIENNES 1968; HERRE and RÖHRS 1973) they are still closely related to the other Canidae. For instance, the domestic dog and the coyote (*Canis latrans*) have the same 78 chromosomes (Mammalian Chromosomes *1*, folio 20 and 21, 1967), and while the jackal (*Canis aureus*) has only 74 chromosomes (MATTHEY 1954) it is still close to the dog, since dog-jackal hybrids are fertile (HERRE 1971; HERRE and RÖHRS 1973). Dog-wolf hybrids are also fertile.

## Materials and methods

The blood from dogs was received from two veterinary clinics, Dansk Dyreværns Internat (Tingskoven) and Århus Kommune hospital's dog-farm. The blood samples from wolf, coyote, jackal, Hallstrom-dog, dog-wolf hybrid, and dog-jackal hybrid were supplied by the Department of Domestic Animals at

*Table 2.* Previous electrophoretic work in Canidae

| Protein | Animals | Results | References |
|---|---|---|---|
| Serum esterases | Dogs and foxes | The zymogram of the dog esterase is different from that of the fox esterase. | KAMINSKY and BALBIERZ 1965 |
| Tetrazolium oxidase | Dogs and coyotes | The dog and the coyote have identical zymograms. | BAUR and SCHORR 1969 |
| Serum proteins<br>Lactate dehydrogenase<br>Glutamic-oxaloacetic transaminase<br>Glutamic-pyruvate transaminase | Dogs, jackals, wolves polar-foxes and coyotes | Only in the serum proteins is it possible to distinguish between the different species. | WASMUND 1966 |
| One quantitative and nine qualitative markers | Dogs, Papuan wild dogs, red foxes, dingos, northern wolf, golden jackal and coyote | No differences between dingos and domestic dogs and coyotes. One difference between dog and northern wolf (transferrins), two between dog and jackal (transferrins and phosphoglucose isomerase), three between red fox and dog (transferrins, albumins and phosphoglucose isomerase). | P. CLARK (pers. comm.) |

*Table 3.* Buffer systems and staining procedures for the enzymes used in this study

| Blood fraction | Protein | Abbreviation | References | |
|---|---|---|---|---|
| | | | Buffer system | Staining procedures |
| Red cells | Acid phosphatase | Ap | GIBLETT 1969 | SØRENSEN 1970 |
| | Adenosine deaminase | ADA | SPENCER et al. 1964 | SPENCER et al. 1968 |
| | Adenylate kinase | AK | FILDES and HARRIS 1966 | FILDES and HARRIS 1966 |
| | Catalase | CAT | KELLY et al. 1971 | SHAW and KOEN 1968 |
| | NADH-diaphorase | DIA | GIBLETT 1969 | GIBLETT 1969 |
| | Esterase | H-EST | POULIK 1957 modified | SIMONSEN and FRYDENBERG 1972 |
| | Glucose-6-phosphate dehydrogenase | G-6-PD | GIBLETT 1969 | GIBLETT 1969 |
| | Glutamic-oxaloacetic transminase | Got | ASHTON and BRADEN 1961 | SCHWARTZ et al. 1963 |
| | Hemoglobin | Hb | LKB note 75 March 29, 1973 | With amidoblack |
| | Lactate dehydrogenase | LDH | FILDES and HARRIS 1966 | HYLDGÅRD-JENSEN 1963 |
| | Malate dehydrogenase | MDH | FILDES and HARRIS 1966 | As LDH with malate as substrate |
| | Nucleoside phosphorylase | NP | SPENCER et al. 1964 | ANSAY and HANSET 1972 |
| | 6-phosphogluconate dehydrogenase | 6-PGD | GIBLETT 1969 | GIBLETT 1969 |
| | Phosphoglucose isomerase | PGI | SPENCER et al. 1964 | YNDGÅRD 1972 |
| | Phosphoglucomutase | PGM | HJORTH 1971 | HJORTH 1971 |
| | Tetrazolium oxidase | To | BAUR and SCHORR 1969 | BAUR and SCHORR 1969 |
| Plasma | Albumin | Alb | DAY et al. 1971 | DAY et al. 1971 |
| | Amylase | AMY | NIELSEN 1969 | NIELSEN 1969 |
| | Esterase | P-EST | KOMMA 1968 | SIMONSEN and FRYDENBERG 1972 |
| | Leucine aminopeptidase | Lap | FILDES and HARRIS 1966 | SHAW and PRASAD 1970 |
| | Transferrins | Tf | ASHTON and BRADEN 1961 or KOMMA 1968 | With amidoblack |

*Table 4.* Dogs used for the investigation
Grouping after MOORE (1962). The numbers in parentheses give the number of dogs, which are parents to the pups

| Group | Breeds | No. of dogs |
|---|---|---|
| I | Alsatian | 40 (8) |
| | Alsatian pups | 44 |
| | Collie | 10 |
| | Old English Sheepdog | 1 |
| II | Cocker Spaniel | 4 |
| | Gordon Setter | 2 |
| | Miniature Poodle | 1 |
| | Pomeranian | 1 |
| | Poodle | 8 |
| | Shorthaired Bird Dog | 3 |
| | Springer Spaniel | 1 |
| III | Airedale Terrier | 3 |
| | Golden Retriever | 3 |
| | Great Dane | 7 |
| | Irish Wolfdog | 1 |
| | Lakeland Terrier | 1 |
| | Shorthaired Dachshund | 1 |
| IV | Boxer | 1 |
| | Doberman Pinscher | 2 |
| | English Bulldog | 1 |
| | French Bulldog | 2 |
| | Labrador Retriever | 12 (10) |
| | Labrador Retriever pups | 28 |
| | New Foundland Dog | 2 |
| | St. Bernhard | 3 |
| | Shih-Tzu | 1 |
| | Mongrels | 14 |
| | Total | 197 |

*Table 5.* Other Canidae than *Canis familiaris* used for this investigation

| Species | No. of animals |
|---|---|
| Wolf, *Canis lupus* | 2 |
| Jackal, *Canis aureus* | 1 |
| Coyote, *Canis latrans* | 1 |
| Hallstrom-dog, *Canis hallstromi* | 2 |
| Red fox, *Vulpes vulpes* | 3 |
| Dog-wolf hybrid | 2 |
| Dog-jackal hybrid | 2 |

Kiel University, Germany. The fox blood samples were taken from red foxes shot in Frijsenborg forest near Århus. In all cases, the samples were taken from a vein in the front leg of the animal and transferred into tubes containing heparin. After arrival at the laboratory, the blood was separated into the plasma and the red blood cell fractions by centrifugation. The blood cells were mixed with ethylene glycol-citrate solution (COOPER et al. 1971) and then both fractions were stored at −18 C until use. Before electrophoresis the red blood cell fraction was centrifuged and the ethylene glycol-citrate solution was pipetted off. The cells were then hemolysed with equal amount of water.

Mostly, electrophoresis was carried out in a horizontal starch gel as described by HJORTH (1971). For the amylase enzyme agar-gel electrophoresis was used, as described by NIELSEN (1969). For phosphoglucose isomerase and hemoglobin, gel electrofocusing in polyacrylamide, pH 3.5—9.5, was used. The method is described in LKB's note no. 75 (LKB-produkter AB, S-161 25 Bromma 1, Sweden, March 29, 1973).

Table 3 lists the proteins studied (with abbreviations), buffer systems and staining procedures. Table 4 lists the dogs used for this investigation, and Table 5 the non-dog Canidae.

# Results

The analysed enzymes and proteins are listed in Tables 6 and 7, which also give the number of dogs analysed and whether the enzyme is polymorphic. Fig. 1 shows the zymograms of the dog-enzymes and compares them with the corresponding human enzymes. If any of the other Canidae differ from the dog zymogram, their zymograms are shown, too.

*Comments to Fig. 1*

Ap — All the animals have identical zymograms, and the zymograms are equal to the heavy zone, described by BRÆND and AUSTAD (1973). The human enzyme has very weak activity and is nearly impossible to score using this method.

CAT — Some dogs have one zone of activity, others none. The enzyme moves just as fast as the human CAT. A cross between a male with activity and a female without activity gave 3 pups without activity and 5 with. Two other crosses between the same male and two females with activity gave 4 pups with activity and 3 without in the first cross and 5 pups with activity and 2 without in the second cross.

H-EST — All the adult dogs have 5—6 bands, and with quantitative variations in the activity of the bands. The pups have only 4 bands. The activity of the dog H-EST is much weaker than the human red blood cell esterase.

*Table 6.* Proteins in hemolysate from dogs
Grouping after MOORE (1962)

| Protein | Group | | | | Mongrels | Sum | Polymorphic |
|---|---|---|---|---|---|---|---|
| | I | II | III | IV | | | |
| Ap | 77 | 12 | 5 | 48 | 8 | 150 | No |
| ADA | 1 | 1 | 2 | 38 | 5 | 47 | No |
| AK | 67 | 8 | 2 | 39 | 6 | 122 | No |
| CAT | 1 | 1 | 2 | 38 | 5 | 47 | Yes |
| DIA | 9 | 6 | 2 | 38 | 6 | 61 | No |
| H-EST | 86 | 18 | 6 | 48 | 9 | 167 | Yes |
| G-6-PD | 25 | 1 | 2 | 38 | 5 | 71 | No |
| Got | 1 | 1 | 2 | 38 | 5 | 47 | No |
| Hb | 95 | 20 | 16 | 52 | 14 | 197 | No |
| LDH | 95 | 20 | 16 | 52 | 14 | 197 | No |
| MDH | 83 | 13 | 5 | 46 | 8 | 155 | No |
| NP | 1 | 1 | 2 | 38 | 5 | 47 | No |
| 6-PGD | 8 | 1 | 2 | 38 | 5 | 54 | No |
| PGI | 28 | 4 | 4 | 41 | 7 | 84 | No |
| PGM | 91 | 19 | 15 | 49 | 14 | 188 | No |
| To | 91 | 14 | 15 | 49 | 13 | 182 | Yes |

*Table 7.* Proteins in plasma from dogs
Grouping after MOORE (1962)

| Protein | Group | | | | Mongrels | Sum | Polymorphic |
|---|---|---|---|---|---|---|---|
| | I | II | III | IV | | | |
| Alb | 1 | 1 | 2 | 38 | 5 | 47 | Yes |
| AMY | 1 | 1 | 2 | 38 | 5 | 47 | No |
| P-EST | 95 | 20 | 16 | 52 | 14 | 197 | Yes |
| Lap | 1 | 1 | 2 | 38 | 5 | 47 | No |
| Tf | 95 | 20 | 16 | 52 | 14 | 197 | Yes |

LDH — All the animals have 5 zones with activity, which are identical for all the animals except the fox. All the Canidae and the human red blood cells have one identical migrating band, suggesting that they might have one subunit with the same mobility, but a change in the second subunit in man and fox.

MDH — All the animals have one band, migrating identically. When stored in a freezer for a month, the band splits up into three bands, one with heavy activity and two with lesser activity. MEERA KHAN et al. (1973) have found MDH in the mitochondrial form to be a dimer. My observation suggests that MDH is also a dimer in blood.

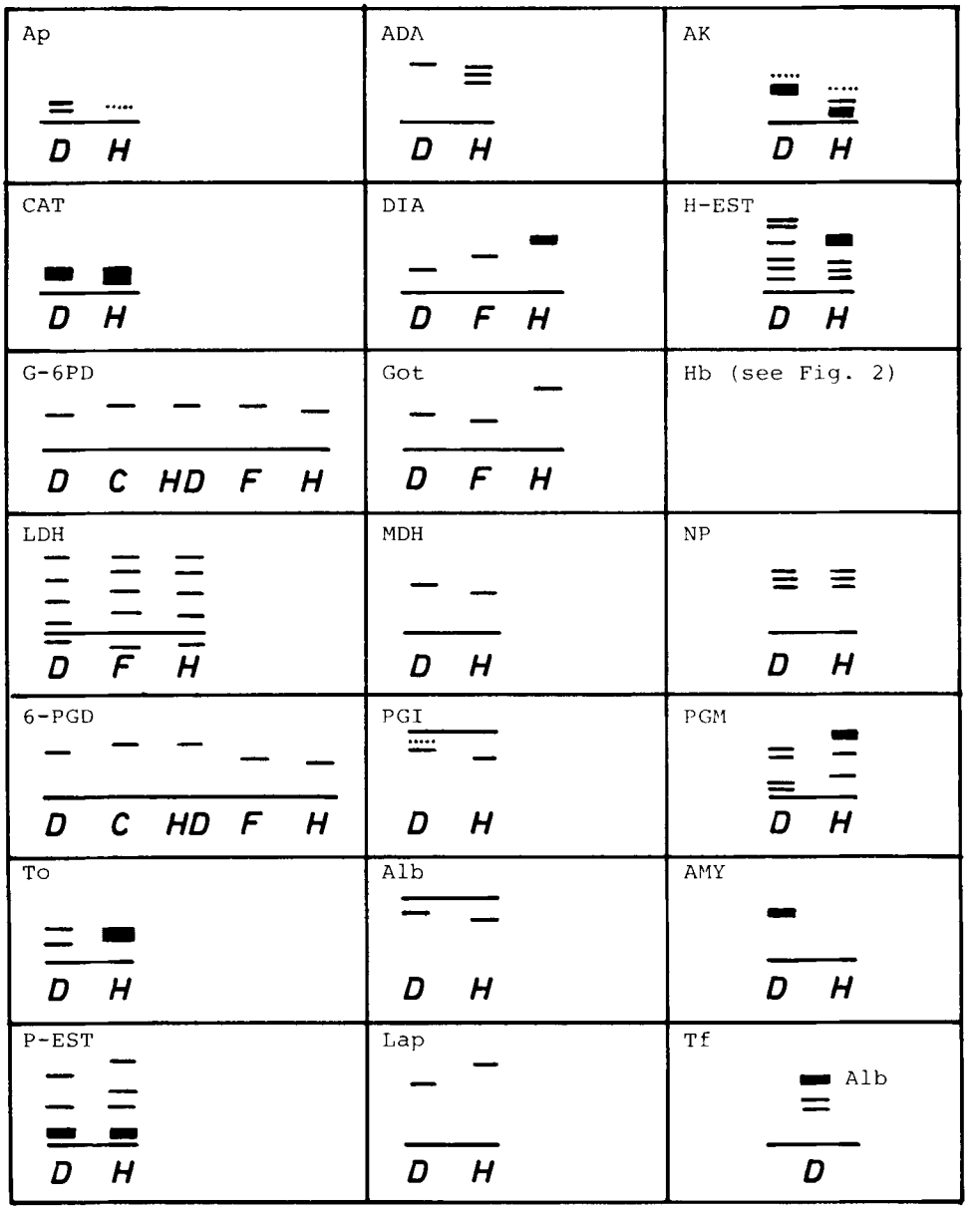
PGI — All the animals have two bands, one heavy and one faint. When using starch gel electrophoresis the migration of the bands of the coyote, the jackal, the fox, and the dog-jackal hybrid moves faster than the PGI from other Canidae, but the difference between the two groups is very small. Using gel electrofocusing, the pattern is much more obvious (see Fig. 2).

To — Among 110 adult dogs there were 4 heterozygotes and 1 rare homozygote, which gives a gene-frequency of 0.955 of the common allele. The other Canidae have all the common allele of the dog To.

Alb — All the dogs have the same electrophoretical mobility of this protein, except one, which was a heterozygote. The other Canidae have the same common allele as the dog.

P-EST — All the dogs have two zones with weak activity and one with heavy activity, corresponding to the human $C_4$ (GIBLETT 1969, p. 205). The heavy zone has at least 3 positions for the band and some of the dogs have two bands. The other Canidae have zymograms which are like those of the dogs.

Tf — The dogs are polymorphic in this protein as described by BRÆND (1966) and by STEVENS and TOWNSLEY (1970). My results only confirmed that




D = dog J = jackal C = coyote
F = fox H = human
DJ = dog-jackal hybrid HD = Hallstrom-dog

Fig. 1. Zymograms of the analyzed enzymes and proteins.

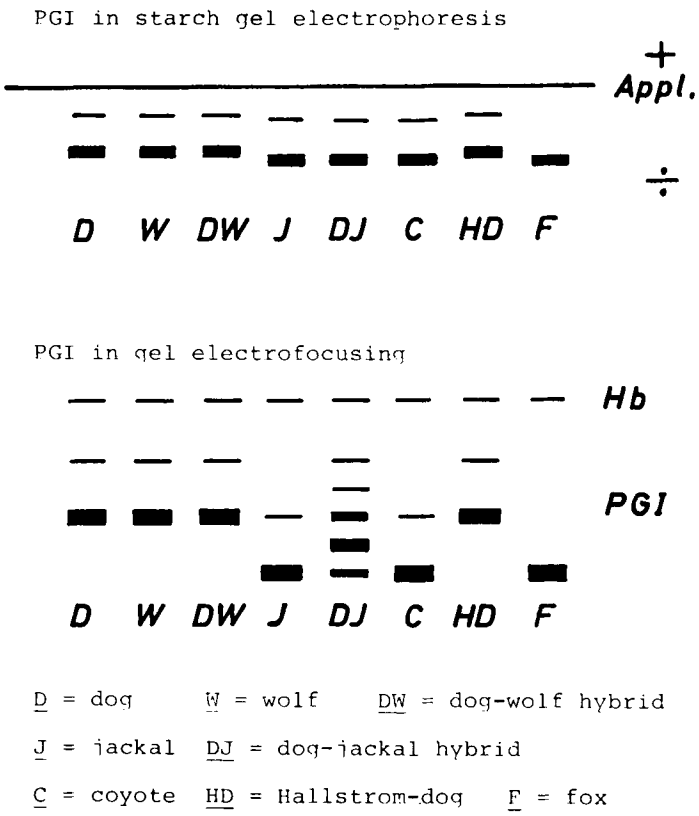



Fig. 2. PGI zymogram of Canidae in starch gel electrophoresis and in gel electrofocusing.

Tf is determined by one locus with three alleles, and the gene-frequency of the common allele is 0.667 in this material.

# Discussion

The results observed in the present work are listed together with the results obtained by MEERA KHAN et al. (1973) and by WEIDEN et al. (1974) in Table 8. Several proteins seem to be polymorphic in dogs. Combining all these studies and the studies listed in Tables 1 and 2, I can summarize the data in dogs and other Canidae as follows:

*Enzymes polymorphic in dogs*

*Acid phosphatase* was found by BRÆND and AUSTAD (1973), one locus with two codominant alleles, gene frequency 0.95.

*Catalase* is described as polymorphic in this study and my results can be explained by the model put forward by ALLISON et al. (1957) as an autosomal locus with two alleles. The male used for the crosses is assumed to be a heterozygote C/c and first mated to a homozygous female c/c, where c/c is inactive in starch gel electrophoresis and C/C and C/c are active but indistinguishable from each other. The two other matings can be explained as C/c × C/c. However, the genetics may be more complex, since FERNSTEIN et al. (1968) and WONG et al. (1972) distinguished different levels of activity and could not explain their results using the simple model put forward by ALLISON et al. (1957).

*Esterase in hemolysate* is polymorphic, but the variation is quantitative. The bands have different activity in the different dogs. I have not been able to explain the genetics, because I have very few crosses in the material, and the pups missed two of the bands compared to the adults.

*Glutamic-oxaloacetic transaminase* is described as

*Table 8.* A comparison between three investigations of red blood cell enzymes in dogs

| Protein | No. of dogs analysed by | | | Variants observed by | | |
|---|---|---|---|---|---|---|
| | MEERA KHAN | WEIDEN | SIMONSEN | MEERA KHAN | WEIDEN | SIMONSEN |
| Acid phosphatase | — | 80 | 150 | — | 1 | 0 |
| Adenosine deaminase | 92 | 47 | 47 | 0 | 0 | 0 |
| Adenylate kinase | 92 | 59 | 122 | 0 | 0 | 0 |
| Aldolase | — | 75 | — | — | 0 | — |
| Catalase | — | — | 47 | — | — | P |
| NADH-diaphorase | 100 | — | 61 | 0 | — | 0 |
| NADPH-diaphorase | 100 | — | — | 0 | — | 0 |
| 2,3-diphosphoglycerate mutase | — | 73 | — | — | 0 | — |
| Enolase | — | 80 | — | — | 0 | — |
| Esterase | — | — | 167 | — | — | P |
| Glucose-6-phosphate dehydrogenase | 92 | 79 | 71 | 1 | 0 | 0 |
| Glutamic-oxaloacetic transaminase (C) | 92 | 110 | 47 | 1 | P | 0 |
| Glutamic-oxaloacetic transaminase (M) | 90 | — | — | 0 | — | — |
| Glyceraldehyde-3-phosphate dehydrogenase | — | 76 | — | — | 0 | — |
| Hemoglobin | — | 82 | 197 | — | 0 | 0 |
| Hexokinase | — | 32 | — | — | 0 | — |
| Isocitrate dehydrogenase (C) | 92 | 80 | — | 0 | 0 | — |
| Isocitrate dehydrogenase (M) | 90 | | — | 0 | | — |
| Lactate dehydrogenase | 92 | 46 | 197 | 0 | 0 | 0 |
| Malate dehydrogenase (C) | 92 | — | 155 | 0 | — | 0 |
| Malate dehydrogenase (M) | 92 | — | | 1 | — | |
| Monophosphoglyceromutase | — | 80 | — | — | 0 | — |
| Nucleoside phosphorylase | — | ? | 47 | — | 0 | 0 |
| Peptidase A | 92 | — | — | 0 | — | — |
| Peptidase B | 92 | 80 | — | 0 | 0 | — |
| Peptidase C | 92 | 80 | — | 0 | 0 | — |
| Peptidase D | 93 | — | — | P | — | — |
| Phosphofructokinase | — | 40 | — | — | 0 | — |
| 6-phosphogluconate dehydrogenase | 92 | 79 | 54 | 0 | 0 | 0 |
| Phosphoglucose isomerase | 92 | 80 | 84 | 0 | 0 | 0 |
| Phosphoglucomutase (1) | 92 | 72 | 188 | 1 | 0 | 0 |
| Phosphoglucomutase (2) | 92 | — | — | 3 | — | — |
| Phosphoglucomutase (3) | 92 | — | — | P | — | — |
| Phosphoglycerate kinase | 92 | — | — | 0 | — | — |
| Pyruvate kinase | — | 26 | — | — | 0 | — |
| Tetrazolium oxidase | 92 | 71 | 182 | P | P | P |

C = cytoplasmic, M = mitochrondrial, P = polymorphic. The ? indicates that the number of dogs is not stated

polymorphic and determined by one locus with two alleles. The Basenji breed is especially polymorphic for this enzyme (WEIDEN et al. (1974)). The gene frequency of the common allele in this breed is about 0.60.

*Peptidase D* is described as polymorphic by SAISON (1972), one locus with two alleles, gene frequency about 0.80; this result was confirmed by MEERA KHAN et al. (1973).

*Phosphoglucomutase 3* was found by MEERA KHAN et al. (1973) and has a frequency of the common allele of about 0.70. It is determined by one locus with three alleles.

*Tetrazolium oxidase* was found by BAUR and SCHORR (1969). The gene frequency ranges from 0.87 to 0.95. It has an autosomal locus with two alleles. The result of BAUR and SCHORR (1969) is confirmed by all three studies referred to in Table 8.

*Albumin, haptoglobin and transferrin* are all polymorphic (see Table 1). *Leucine aminopeptidase* is also polymorphic with a gene frequency of 0.97, found by TANABE et al. (1974). The present work adds *esterase in plasma* to the list of polymorphic enzymes in dogs.

With these data it is possible to give provisional answers to the three questions posed in this study. The first question was to estimate the level of electrophoretic variation in domestic dogs. Of the 46 presumptive loci investigated 12 were found to be polymorphic (26%). This is similar to the level of electrophoretic variation found in other mammals (FRYDENBERG and SIMONSEN 1973). The gene frequency of the common allele in the polymorphic enzymes is high in each case, so that the level of genic heterozygosity is low. This is also common for mammals (FRYDENBERG and SIMONSEN 1973).

The second question was, if there are any effects at the protein level of the selection to which dogs have been subjected by man. The present work has found no discrete variation between breeds or groups of breeds so that the effects of selection seem to have been small at the protein level. It is still possible, however, that if a large number of dogs from each breed were screened for the polymorphic enzymes, differences in gene frequencies might emerge similar to those found by TANABE et al. (1974). Evidence that differences between dogs do exist on the protein level is provided by LEONE and ANTHONY (1966) who also used immunoelectrophoretic techniques on serum esterases.

The last question was to see whether isoenzymes can be used to clarify the taxonomic relationships between domestic dogs and their relatives. The differences between the Canidae are shown in Fig. 1 and Fig. 2. I have only used the enzymes which are identical in all dogs. No differences between the enzymes of the dog, of the wolf and of the dog-wolf hybrid were observed. Only one difference between the dog, the jackal and the dog-jackal hybrid, namely with regard to PGI, was found (see Fig. 2). The Hallstrom-dog and the coyote have identical G-6-PD and 6-PGD enzymes, which, however, are not identical to the dog's enzymes. Furthermore the coyote has a PGI enzyme similar to the jackal's PGI. Six of 14 enzymes observed in the fox have zymograms differing from those of the dog. The G-6-PD of the fox is identical with the G-6-PD of the coyote and the Hallstrom-dog, and the main fraction of the PGI of the fox corresponds completely to the PGI of the jackal and of the coyote.

These results indicate that the dog and the wolf are very closely related, and may have a common ancestor. By using multiple character analysis LAWRENCE and BOSSERT (1967) have obtained evidence that the dog and the wolf are closely related. The jackal seems to be less closely related to the dog than the wolf. VRIESENDORF (1972) has hypothesized that the dog and the wolf have a common ancestor. This theory is based on the analysis of the dog histocompability antigens found in other Canidae than dog. The Hallstrom-dog and the coyote have electrophoretic identical enzymes, except PGI. SCHULTZ (1968) has put forward the hypothesis, that the Hallstrom-dog is a dog returned to a wild state and mixed up with other Canidae. The author's measurements of skull, skeleton etc. strongly indicate that the Hallstrom-dog is closely related to the dingo. P. CLARK (pers. comm.) has not found any difference between dog and dingo (see Table 2). The fox seems to be more closely related to the jackal and to the coyote than to the dog and the wolf.

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