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Genetic Polymorphism of Tetrazolium Oxidase in Dogs

Abstract. *Two alleles,* To^A *and* To^B, *determine a genetic polymorphism of tetrazolium oxidase in canine erythrocytes. The approximate gene frequencies for most dog breeds are 0.94 for* To^A and *0.06 for* To^B. *The gene frequencies of the German shepherd, 0.8 for* To^A *and 0.2 for* To^B, *differ significantly from those of other breeds. The electrophoretic tetrazolium oxidase isozyme pattern of* Canis latrans *closely resembles the tetrazolium oxidase A pattern of* Canis familiaris.

Brewer demonstrated tetrazolium oxidase in several human tissues and classified the enzyme as an indophenol oxidase (*1*). The exact physiological role of this oxidase is obscure. Its ability to catalyze the transfer of electrons from reduced *p*-nitro blue tetrazolium to oxygen without the presence of a coenzyme suggests a way to detect its site of action after electrophoresis. Unlike the tetrazolium oxidase from human erythrocytes, which presents a common isozyme pattern throughout the species except for rare genetic variants (*1, 2*), the canine oxidase exhibits a genetic polymorphism determined by two alleles, To^A and To^B.

Blood samples were obtained from 189 dogs of various breeds selected at random at a veterinary clinic. Forty-three animals of a beagle colony (*3*), two coyotes (*Canis latrans*), and one coyote-dog hybrid were also tested. In addition, blood samples from 42 offspring of beagles heterozygous at the *To* locus were examined. Fresh blood obtained by venipuncture was absorbed on Whatman 3 MM filter paper (4 by 15 mm). The blood-impregnated paper strips were dried at room temperature and stored at below −20°C. The paper strips were applied to thin-layer starch-gel (1 mm), and electrophoresis (*4*) was performed at 130 volts for 16 hours at 0°C in a gel buffer (*p*H 9.1) containing 0.1*M* tris(hydroxymethyl)aminomethane (tris), $3.25 \times 10^{-3}M$ ethylenediaminetetraacetate (disodium salt), and $1.5 \times 10^{-2}M$ boric acid. The electrode compartments contained 0.3*M* sodium borate buffer, *p*H 8.8. The gels were rinsed for 3 minutes in 0.3*M* sodium borate buffer, *p*H 8.5, and then stained for 2 to 3 hours in daylight in 100 ml of 0.1*M* tris-HCl buffer (*p*H 8.5) containing 15 mg of *p*-nitro blue tetrazolium (Sigma), 15 mg of phenazine methosulfate, and 20 mg of magnesium chloride at 37°C. The stained gels were submerged in a mixture of methanol, acetic acid, and water (5:1:5) for 2 minutes, placed in an aqueous solution of glycerol (15 percent by volume) and acetic acid (2 percent by volume) for 30 minutes, and plasticized at 50°C (*4*).

Canine tetrazolium oxidase, like that from humans and most other mammals, separated on starch gel into many fractions. Isozyme patterns of whole blood and of red cell lysate prepared by lysing erythrocytes that were washed three times with distilled water were identical. The most frequently observed isozyme pattern, type A, consisted of three to four achromatic zones at the anodic side of the gel (Fig. 1). Type A was observed in 78 males and 84 females of the 189 dogs from the clinic series. Type A was also found in 36 (19 male, 17 female) of 43 beagle blood samples. The B isozyme pattern, which resembled the A pattern except for a more cathodic displacement of all tetrazolium oxidase zones, was found only in one dog, a wirehaired fox terrier. The remaining blood samples from the clinic series (14 male and 12 female) and three male and four female samples from the beagle colony exhibited a more complex zonal pattern AB (Fig. 1). The electrophoretically fastest and slowest zones of the AB pattern resembled in mobility the A and B fractions, respectively. In between the A and B regions and partially superimposed on them lay additional achromatic zones. The AB pattern suggested that such individuals synthesized both the A and B tetrazolium oxidase isozyme series and also a series of molecular hybrid isozymes of intermediate electrophoretic mobility, altogether probably as many as 12 isozymes of the oxidase. The canine heterozygous tetrazolium oxidase pattern appeared to be analogous to the oxidase isozyme pattern observed in human heterozygotes (*2*). The oxidase isozyme pattern of the two coyotes and one hybrid animal were electrophoretically indistinguishable from the canine oxidase type A.

The zymogram patterns suggested the presence of two codominant alleles at the canine *To* locus. The codominant transmission of the *To* character was confirmed by nine backcross matings which produced 20 A and 22 AB offspring. This result agrees with the expected ratio of 1:1 (Table 1). Transmission of the B gene from father to son was observed four times, indicating

Table 1. The *To* backcross matings of beagles.

Litters (No.)	Whelps (No.)*			
	Male AA	Female AA	Male AB	Female AB
	Sires AA × dams AB			
5	3	5	3	10
	Sires AB × dams AA			
4	6	6	4	5

* Survivors of litters 4 to 10 months old.

Table 2. The *To* gene frequencies in the dog.

Breed	Dogs (No.)	Heterozygotes (No.)	Allele A	Allele B
Beagle colony, a	43	7	0.919	0.081
German shepherd, b	20	8	0.800	0.200
Terriers, c	14*	1	0.893	0.107
Cocker spaniel, d	13	1	0.962	0.038
Poodle, e	19	1	0.974	0.026
Dachshund, f	21	1	0.976	0.024
Miscellaneous, g	94	12	0.936	0.064
Pooled, c–g	161	16	0.944	0.056

* Includes one B homozygote.

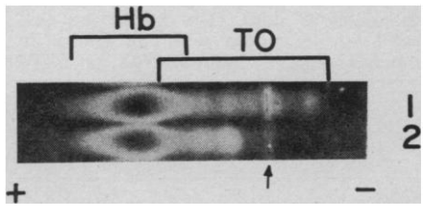

Fig. 1. Thin-layer starch-gel electrophoresis of canine whole blood at *p*H 9.1, stained for tetrazolium oxidase (TO). The arrow indicates the origin. Zymogram *1*, heterozygote AB; zymogram *2*, homozygote A. The most anodic zones of the oxidase are not recognizable because the hemoglobin (Hb) band interferes. There is also superimposition of tetrazolium oxidase bands in the heterozygous blood sample.

Table 3. Observed and expected canine tetrazolium oxidase genotype frequencies. Genotype frequencies calculated according to the Hardy-Weinberg law are shown in parentheses below the observed frequencies. The last column gives the probabilities *P* of the chi-square tests for agreement between observed and expected frequencies.

No. of dogs with genotype			*P* (2 d.f.)
AA	AB	BB	
	Clinic series		
144	16	1	
(143.50)	(17.00)	(0.50)	>.70
	Beagle colony		
36	7	0	
(36.28)	(6.43)	(0.28)	>.80
	German shepherds		
12	8	0	
(12.80)	(6.40)	(0.80)	>.30

autosomal inheritance of the canine *To* locus.

The *To* gene frequencies of dog breeds (crossbreeds excluded) are presented in Table 2. A chi-square test on the four groups, a, b, c to f, and g of Table 2 indicated significant heterogeneity in gene frequency among breeds ($\chi^2 = 11.3149$, d.f. $= 3$, $P < .025$), Though the gene frequencies for most breeds were relatively close to each other, the German shepherd breed made a significant exception. Eight of twenty German shepherds and two of eight German shepherd hybrids were heterozygous at the *To* locus. The remaining 16 purebred heterozygous animals were found in poodle, cocker spaniel, dachshund, chihuahua, terrier, boxer, huskie, sheltie, collie, Irish setter, and beagle breeds. Similar gene frequencies in diverse canine breeds might suggest a balanced *To* polymorphism maintained by some selective advantage of the heterozygote. If a selective advantage was indeed present, it was not apparent from our data because the observed heterozygote frequencies in the various groups listed in Table 3 agreed closely with the frequencies expected on the basis of the Hardy-Weinberg equilibrium. The absence of a significant heterozygote advantage is not surprising in a relatively small population sample such as the present.

Genetic drift may account for the high To^B gene frequency in the German shepherd. The German shepherd, first intensively bred at the turn of the century in Germany and two decades later in America (*5*), may carry a disproportionately large amount of genes from a relatively few champion sires. As an alternative explanation, the high To^B gene frequency of the German shepherd may represent an adaptation to a different internal environment.

Gene frequency data on canine species other than *C. familiaris* are not available for comparison. Such comparative biochemical information could possibly elucidate the obscure phylogenetic origin of the dog and its exact relation to other species of *Canis*.

A survey which included blood samples from 83 mammalian species showed that tetrazolium oxidase isozymes of several species of a genus frequently possessed identical electrophoretic mobilities, for example, *Macaca fuscata, M. irus, M. speciosa, M. mulatta,* and *M. nemestrina; Microtus richardsoni, M. oregoni, M. montanus, M. longicaudatus,* and *M. ochrogaster*; and *Felis catus* and *F. concolor*. The oxidase of *Pan troglodytes* and man are also electrophoretically indistinguishable from each other. Electrophoretical comparison, however, cannot exclude electrophoretically mute variations. Multiple erythrocyte isozymes of this oxidase seemed to be the rule in mammals. Except for the dog, genetic polymorphism was not observed in multiple blood samples of several mammalian species.

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Amino Acid Uptake by Kidney and Jejunal Tissue from Dogs with Cystine Stones

Abstract. *Cystine and lysine accumulation in vitro in intestinal and renal tissue was studied in eight dogs that form cystine stones. Under conditions which demonstrate in vitro defects in tissue obtained from humans with cystinuria, normal amino acid accumulation occurred in six dogs with the canine disorder. Normal amino acid uptake in tissue and the demonstration of normal to minimum increases in excretion of lysine suggest that the canine disorder is not similar to the human syndrome.*

There has been a continued effort to find animal models for human disease (*1*). The occurrence of cystinuria with calculi in dogs has led to several reports indicating that the canine disorder may be considered a counterpart of human cystinuria. Brand, Cahill, and Kassell (*2*) attempted to breed a line of cystinuric Irish terriers for investigation of cystine metabolism. When the human condition was shown to be associated with an increased renal clearance of cystine plus the dibasic amino acids, lysine, arginine, and ornithine, there followed several studies of urinary amino acids in the canine disorder. Affected dogs excrete large quantities of cystine with normal amounts of cystine in the plasma, an occurrence which parallels the human abnormality (*3*). Dibasic amino acids have been observed in the urine of cystinuric dogs (*4*, *5*), but the excretion was variable; some affected dogs had solely cystine in the urine, while others had only cystine and lysine without arginine or ornithine. These findings differ from those found in the homozygote human condition. As a result of lysine feeding experiments, the existence of an intestinal transport defect in these dogs similar to that seen in human cystinuria was postulated (*6*).