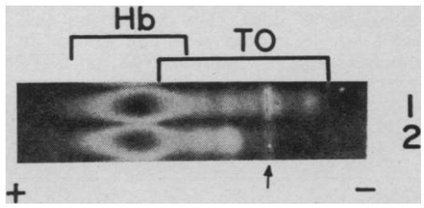

Fig. 1. Thin-layer starch-gel electrophoresis of canine whole blood at *p*H 9.1, stained for tetrazolium oxidase (TO). The arrow indicates the origin. Zymogram *1*, heterozygote AB; zymogram *2*, homozygote A. The most anodic zones of the oxidase are not recognizable because the hemoglobin (Hb) band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

ERNST W. BAUR
Division of Research, P.O. Box 94008, Fort Steilacoom, Washington 98494
RICHARD T. SCHORR
Jones Animal Hospital, 3322 South Union, Tacoma, Washington 98409

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6. We thank Drs. J. Garner and A. C. Lee for the beagle blood samples and pedigree data of the animals, Dr. M. L. Johnson for the coyote blood samples, and Mrs. J. Tomita for technical assistance.

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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*).

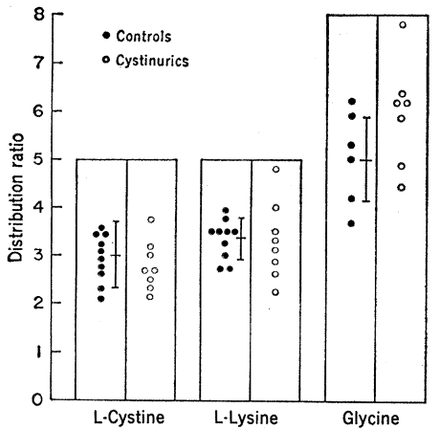

Fig. 1. The uptake of amino acids by kidney cortex slices from cystinuric dogs. Kidney cortical slices (3 to 10 mg) made from surgical biopsy specimens were incubated at 37°C for 30 minutes in flasks containing 0.2 μc of radioactive amino acids (0.07 m*M*) in 2 ml of Krebs-Ringer-bicarbonate buffer (*p*H 7.4). Assay of intracellular amino acids and calculations of the distribution ratio were performed as previously described (*7*). Mean ± 1 S.D. for controls are shown.

Heterogeneity in human cystinuria has been demonstrated by the variable uptake of lysine and cystine by jejunal and kidney tissue from cystinuric patients. Cystine accumulation in kidney slices from human cystinuric patients is not different from that in normal controls. However, lysine and arginine accumulation by cystinuric kidney tissue is approximately 50 percent of the uptake in nonaffected kidney slices (*7*). Rosenberg *et al.* (*8*) described three distinct biochemical types of human cystinuria based on the characteristic of lysine and cystine accumulation by jejunal mucosa. Jejunal mucosa from the majority of human cystinurics was unable to maintain a gradient of lysine or cystine (type 1). Tissue from two patients was able to maintain a slight gradient for cystine, but not for lysine (type 2). In type 3 patients there was a variable gradient for both lysine and cystine which was close to normal.

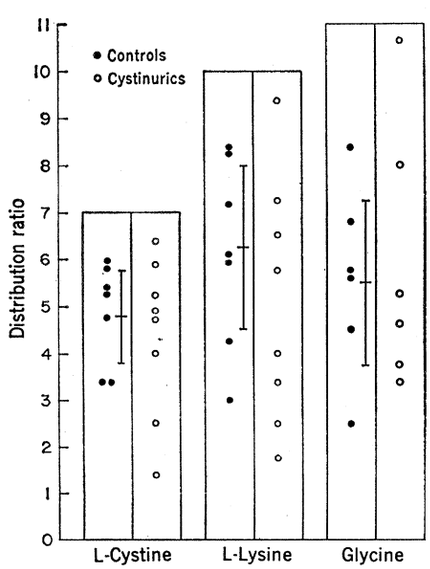

Fig. 2. The uptake of amino acids by intestinal mucosal biopsies obtained at laparotomy. Conditions as in Fig. 1 except amino acid concentration was 0.57 m*M*. Mean ± 1 S.D. for controls are shown.

Using kidney and small intestine biopsy material, we studied cystine and lysine transport characteristics in eight dogs that form cystine stones. We used a method similar to that used in the elucidation of human tissue transport systems. To determine amino acid accumulation by jejunal mucosa and kidney cortical slices, radioactive lysine, cystine, and glycine were employed at conditions identical to those used with human tissue (*7*, *9*). Tissue uptake was expressed as the distribution ratio of the amino acid, that is, the ratio of concentration in the intracellular fluid to the concentration of the same amino acid in the media (*10*). Quantitation of amino acid excretion in the urine was determined on a Spinco model 120 C amino acid analyzer, and urinary cystine was determined by electrolytic reduction (*11*).

The range of cystine excretion in the urine of eight dogs with cystine calculi and normal amounts of cystine in the plasma is shown in Table 1. We did not find a clearly definable lysinuria. Increased excretion of arginine or ornithine was not found in any of the cystinuric dogs. In two dogs in which pure cystine calculi were found, cystine excretion was within the normal range, thus indicating there may be other factors, separate from the quantity of cystine excreted, responsible for the development of cystine stones in these dogs.

The ratios of cystine, lysine, and glycine accumulated in tissue after 30 minutes of incubation for both kidney slices and jejunal mucosa is shown in Figs. 1 and 2. The uptake of glycine is measured as a control because its accumulation is thought not to be mediated by the dibasic amino acid transport system. In the kidney cortical slices from the dogs forming stones, cystine and lysine uptake was essentially normal. Analyses of jejunal mucosa from two dogs demonstrated a slight reduction in uptake of lysine and cystine as compared to our normal range. The other six dogs had normal lysine and cystine uptake. There was no correlation between the amino acid excretion and accumulation in vitro of amino acids in the tissues in any dog.

Table 1. Amino acid excretion of normal and cystinuric dogs. The results are expressed as the mean with the range in parenthesis. Not determined, N.D.

Breed	Calculi (% cystine)	Cystine (mg/g of creatinine)	Lysine (mg/g of creatinine)
Mongrel controls (6)		53 (28–120)	32 (0–36)
Mongrel	86	217	76
Dachsund	77	159	58
Great Dane	95	448	51
Mongrel	92	452	34
Dachsund	100	106	13
Dachsund	96	383	10
Mongrel	73	N.D.	N.D.
Beagle	100	94	5

The finding (i) of normal cystine amounts in the plasma, (ii) of normal cystine accumulation by kidney cortical slices, and (iii) of elevated urinary cystine excretion in dogs is identical to the findings of Fox *et al.* (*7*) in their elucidation of kidney transport characteristics in human cystinuria. When the characteristics of transport in the intestinal mucosa are compared, none of the dogs studied showed complete inability to accumulate cystine or lysine as seen in the type 1 or 2 human cystinuria. All of our dogs essentially resemble the type 3 human cystinuria, in which there is a variable impairment of cystine and lysine uptake, with some of the patients having a normal ability to accumulate cystine and lysine in the intestinal mucosa (*8*). Failure to find intestinal transport defects for cystine in our dogs makes the canine disease unlike most of human cystinuria.

From data on the excretion of amino acids in the urine as well as that on the accumulation of amino acids in the tissues, it appears that heterogeneity exists in the nature of canine cystinuria. Since human cystinuria is also a heterogeneous disorder, correlations between the findings in dogs and humans must be made with caution. The canine disorder may be similar to the cystinuria without dibasic amino aciduria in a family described by Brodehl (*12*).

The independence of the canine kidney cystine transport system from

the dibasic amino acid system is suggested in these studies. This conclusion can be made from human and rat kidney experiments (*13*), as well as from the cystinuria without lysinuria (*12*) and dibasic amino aciduria without cystinuria in humans (*14*). The dog may be a good model for the study of the etiology of excessive urinary cystine excretion. The mechanism of cystinuria which is enigmatic in humans may be studied more easily in the isolated cystinuric situation as it exists in the canine disorder.

PHILIP G. HOLTZAPPLE
Division of Biochemical Development and Molecular Diseases, Children's Hospital of Philadelphia and *Department of Pediatrics, Medical School of the University of Pennsylvania, Philadelphia*
KENNETH BOVEE
Department of Medicine, School of Veterinary Medicine, University of Pennsylvania
CLAIRE F. REA
STANTON SEGAL
Division of Biochemical Development and Molecular Diseases, Children's Hospital of Philadelphia and *Department of Pediatrics, Medical School of the University of Pennsylvania*

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Structural Studies on Transfer RNA: Crystallization of Formylmethionine and Leucine Transfer RNA's

Abstract. *Improved solvent systems were used to crystallize two different transfer RNA species. These crystals show increased mechanical and thermal stability over crystals obtained previously from a similar system. They have sufficient stability and crystalline order to be used in x-ray crystallographic studies.*

With the vapor equilibration technique (*1*), single crystals of transfer RNA (tRNA) with improved thermal and mechanical stability and higher crystalline order were grown. With aqueous ammonium sulfate as the precipitating agent, two different tRNA's were crystallized. These are leucine (Leu) tRNA from *Escherichia coli* and formylmethionine (fMet) tRNA from yeast. The *E. coli* $tRNA^{Leu}$ and the yeast $tRNA^{fMet}$ crystals were used for x-ray diffraction studies (*2*). Both of these tRNA's have been crystallized from eight solvent systems differing only in the nature of the polyvalent cation present.

The initial conditions for crystallization were 0.05*M* NH_4Cl, 0.005*M* sodium cacodylate *p*H 6.0, 0.005 to 0.0075*M* $MgCl_2$, 0.001 to 0.002*M* polyvalent cation, and 30 percent saturated ammonium sulfate. The initial tRNA concentration was between 4 and 10 mg/ml. The polyvalent cations used were spermine, spermidine, Hg^{2+}, Co^{2+}, Mn^{2+}, Cr^{3+}, Cu^{2+}, and Mg^{2+}. In all cases the chloride salts were used. The above sample (final volume, 10 μl) was then equilibrated in a sealed chamber containing a large and precisely controlled solvent reservoir of aqueous ammonium sulfate between 35 and 65 percent saturation, depending on the species of tRNA to be crystallized and on the temperature of the experiment. All concentrations of ammonium sulfate are expressed in terms of percent saturation at 8°C.

Crystals of *E. coli* $tRNA^{Leu}$ were obtained at 8°C from samples equilibrated against 35 to 40 percent saturated ammonium sulfate (*3*). The initial tRNA concentration was 10 mg/ml. A light precipitate forms in this system within 24 hours and crystals appear within 3 days to 4 weeks. The crystals are tetragonal bipyramids (Fig. 1, a and b). The type of polyvalent cation in no way affects the morphology of the crystals. The density of these crystals was 1.48 ± 0.02 mg/cm^3, as determined by use of a density gradient with carbon tetrachloride and cyclohexane. As judged by x-ray data (*2*), the unit cell is tetragonal with dimensions 46 by 46 by 137 Å. If the partial specific volume for tRNA is 0.53 ml/gm (*4*), the crystals are about 43 percent RNA by volume and contain four molecules per unit cell. These crystals show a strong extinction when viewed between crossed polaroids only when observed across the unique axis (Fig. 1c). The extinction is virtually absent when viewed down the unique axis.

X-ray diffraction indicates that there is a twinning in the crystal structure. Although the crystal quality needs to be improved, the diffraction patterns now available are of sufficient quality to provide information about the structure (*2*).

Crystals of yeast $tRNA^{fMet}$ were grown at 8°C and at room temperature. The sample was prepared as described (*5*) and was 90 to 95 percent pure as judged by amino acid acceptor activity. The crystals appear to be identical under both of the growth condi-

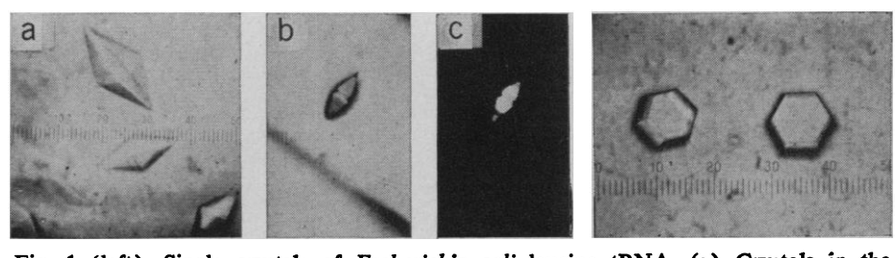

Fig. 1 (left). Single crystals of *Escherichia coli* leucine tRNA. (a) Crystals in the form of tetragonal bipyramids grown with 0.002*M* Hg^{+2} as the polyvalent cation. (b) Single crystals mounted in a quartz capillary ready for x-ray diffraction. (c) Same crystals as in (b) as seen between crossed polaroids. Scale, 0.02 mm per division in all photomicrographs. Fig. 2 (right). Single crystals of yeast methionine tRNA (formylatable) in the hexagonal prism form. These crystals were grown in the presence of 0.0015*M* spermine as the polyvalent cation. Scale, 0.02 mm per division.