3. ———, *ibid.*, plates 5–12.
4. The geomorphology and stratigraphy of the Budiño site are described in K. W. Butzer, *Eiszeitalter Gegenw.*, in press.
5. For further critical and partly controversial opinions on the Asturian of northern Spain, see M. Crusafont-Pairó, *Speleon* (Oviedo) **14**, 77 (1958); F. Jordá-Cerdá, (Actos) *V. Congreso Arqueológico Nacional* (Zaragoza, 1959), pp. 63–66; and ———, *Memorias, Servicio de Investigaciones Arqueológicas* (Diputación Provincial de Asturias, Oviedo, 1958), vol. 3, pp. 1–97.
6. Field work supported in part by a grant-in-aid from the Wenner-Gren Foundation for Anthropological Research (E. de Aguirre) and NSF grant GS-17814 (F. C. Howell). Sedimentological studies and radiocarbon dates were made possible by a second Wenner-Gren grant (K. W. Butzer). We are indebted to P. Biberson (Musée de l'Homme, Paris) and L. G. Freeman (University of Chicago) for valuable discussion.

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Autosomal Phosphogluconic Dehydrogenase Polymorphism in the Cat (Felis catus L.)

Abstract. *Three patterns of 6-phosphogluconic dehydrogenase activity were obtained by starch-gel electrophoresis of blood from domestic cats. Genetic analysis indicates control of these patterns by a pair of alleles at an autosomal locus. Presence of three enzymatically active bands in heterozygotes and of single bands in homozygotes is compatible with at least a dimeric structure for the enzyme.*

Electrophoretic enzymatic variants in diploid organisms were reviewed by Shaw (*1*). No reports on species of the order Carnivora exist. We now report the findings of polymorphism of 6-phosphogluconic dehydrogenase (6-PGD) activity in hemolyzates of blood from domestic cats (*Felis catus* L.) and patterns from single individuals of four other species of Felidae. The manner of inheritance for these variants has been determined in the domestic cat by controlled breeding (*2*).

Blood for electrophoresis was collected into an anticoagulant mixture of acid, citrate, and dextrose with 2.4 g of inosine per 100 ml of solution (*3*). For electrophoresis (*4*), a hemolyzate was prepared by freezing and thawing one part of blood mixed with two parts of water. Horizontal starch-gel electrophoresis was carried out in a tris-ethylenediaminetetraacetate-boric acid buffer (*5*) as described by Porter *et al.* (*6*). Cell-free hemolyzates were placed in slots in the gel with filter-paper wicks. Protein separations were accomplished by running the gel at 4°C overnight (16 to 18 hours) at 350 volts with 25 ma of current. At *p*H 8.6 in this buffer system, the 6-PGD migrates toward the anode.

The gels were sliced horizontally for staining of the cut surface by a modification (*7*) of the method of Fildes and Parr (*8*). A square of Whatman No. 1 filter paper was cut to fit the surface of the sliced gel, and stain was poured over the filter paper. Incubation for 1 to 2 hours at 37°C gave clearly delineated bands of 6-PGD activity. Fixation was by soaking in 50 percent methanol for 2 to 3 days, and gels were stored by wrapping in polyvinyl chloride sheeting.

Cats showed either a single fast band (phenotype A), a single slow band (phenotype B), or three bands (phenotype AB) which correspond in mobility to both the fast and slow band with an additional band of intermediate mobility. A mixture of bloods with A and B phenotype produced A and B bands without an intermediate third band (Fig. 1a).

Among the Felidae other than the domestic cat, single specimens of blood were obtained for each of the species *Panthera tigris* L. (tiger), *Panthera pardus* L. (panther), *Felis rufa* Schreber (bobcat), and *Felis lynx* L. (lynx). All these, except the tiger, which was newborn, were adults. The 6-PGD zymograms for these species and two of the three variants in domestic cats (Fig. 1b) show that, in the blood of tigers, panthers, and lynxes, active components migrate at a much greater rate than in that of the domestic cat. All bands from the Felidae show migration rates greater than those of the usual human 6-PGD type.

The genetics of the 6-PGD patterns in domestic cats were defined by test matings of animals with known 6-PGD phenotype. The total number of matings for each cross (Table 1) includes reciprocal crosses of each appropriate type except in the "A by B" mating. In this group, all dams were of the B phenotype, and all sires were of the A phenotype. Distribution of offspring phenotypes from reciprocal crosses were consistent with single-locus, two-allele controlled inheritance, and therefore the groups were combined. Single bands, either A or B, resulted from apparent homozygosity for the alleles at the locus involved. The AB (three-band) pattern was found in the corresponding heterozygotes. The frequencies of each phenotype from the different matings with adequate numbers do not deviate significantly from the expected frequencies. This manner of inheritance is the same as that described for all other animals examined, except for *Drosophila* in which a sex-linked 6-PGD was found (*9*).

Table 1. Results of controlled matings for genetic analysis of 6-PGD inheritance. From the phenotypes of 110 offspring, inheritance is determined to be autosomal with control by two alleles, Pgd^A and Pgd^B, at the locus. Abbreviations: M, male; F, female.

Matings	Phenotypes of offspring (No.)					
	A		AB		B	
	M	F	M	F	M	F
A × B			4	7		
A × A	3	4				
AB × A	4	3	7	5		
AB × AB	4	1	12	12	3	5
AB × B			7	10	7	9
B × B					1	2

Because a standard nomenclature for the genotype of the 6-PGD system is

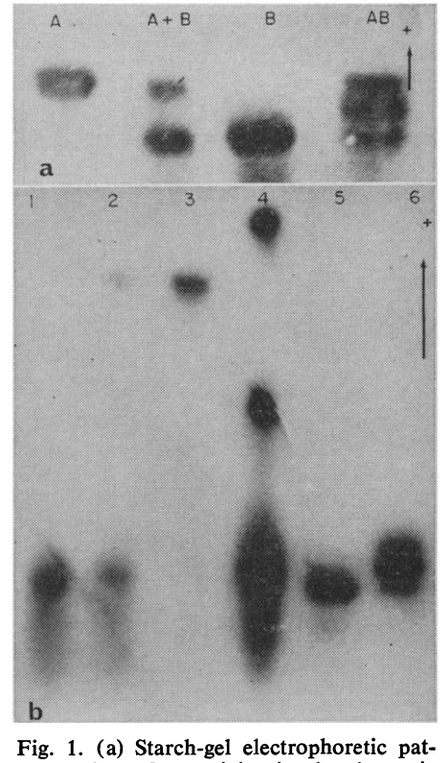

Fig. 1. (a) Starch-gel electrophoretic patterns of 6-PGD activity in the domestic cat, showing the A, A + B mixture, B, and AB phenotypes. The lack of an intermediate band when blood of A and B phenotypes are mixed for electrophoresis contrasts with the AB phenotype pattern and supports polymeric structures for the active enzyme. (b) The four species of Felidae and two cat phenotypes are: 1, bobcat; 2, lynx; 3, panther; 4, tiger; 5, domestic cat B; and 6, domestic cat AB.

lacking, we have chosen to follow Young (*10*) in using the symbols Pgd^A and Pgd^B for the alleles at the 6-PGD locus in the cat. This conforms to standard genetic usage, is conveniently similar to the common symbol used for the enzyme (6-PGD), and is consistent with the more general use of Roman (rather than Greek) letter superscripts. With this nomenclature, the genotypes of cats for 6-PGD variants are Pgd^A/Pgd^A (phenotype A), Pgd^B/Pgd^B (phenotype B), and Pgd^A/Pgd^B (phenotype AB).

The manifestations of activity in three bands in heterozygotes is consistent with the hypothesis of molecular hybridization advanced by Schwartz (*11*) to explain the same situation for an esterase of maize. This has been accepted and extended by others (*12*). According to this hypothesis, each allele controls production of a specific protein subunit, the active enzyme being a dimer. In heterozygotes, random association of subunits occurs and therefore gives rise to twice as many hybrid molecules as homologous molecules of each type in the ratio of $A^2 + 2AB + B^2$. The more intense enzymatic activity of the intermediate band on zymograms is presumably related to the greater number of hybrid enzyme molecules expected by this hypothesis.

In our studies of cats, we noted the greater intensity of enzymatic staining for the intermediate band of the AB phenotype. If the following assumptions are accepted, certain conclusions may be drawn. The assumptions are: (i) both alleles at the 6-PGD locus produce subunit molecules of equal enzymatic efficiency when combined in a dimer; (ii) both alleles are equally active with respect to production of subunits; (iii) polymerization of the subunits to an enzymatically active dimer takes place within the producing cell; (iv) the staining reaction in zymograms is stoichiometrically related to the number of active enzyme molecules. The conclusions would be that gene activity is quantitatively expressed by enzyme activity, that the relative staining intensity of zymogram bands depicts quantitative molecular relationships, and that, in the cat 6-PGD system, the evidence is against differential activity of loci on the two autosomes. Our results (Fig. 1) are consistent with this interpretation.

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References and Notes

1. C. R. Shaw, *Science* **149**, 936 (1965).
2. The cats originally studied and used in genetic analysis were members of a colony established at Rainier School.
3. This mixture is used in preference to one without the inosine when a delay between drawing and laboratory procedures is expected.
4. Electrophoresis was performed in a laboratory of the Department of Medicine, Division of Medical Genetics, Univ. of Washington.
5. Stock buffer contained (per liter) 109 g of tris(hydroxymethyl)aminomethane, 30.9 g of boric acid, 7.6 g of EDTA disodium salt. A 1:20 dilution of stock buffer was used for preparing a gel of 33 g of hydrolyzed starch (Connaught Medical Research Laboratories, Toronto) in 300 ml of diluted buffer. The mixture was heated to 75°C with constant stirring and debubbled by decreased pressure; 0.15 ml of a solution (10 mg/ml) of triphosphopyridine nucleotide was added before pouring the gel in trays (14 by 22 cm). Buffer for the filter-paper contact bridges was stock-diluted 1:7.
6. I. H. Porter, S. H. Boyer, E. J. Watson-Williams, A. Adam, A. Szeinberg, M. Siniscalco, *Lancet* **1964-I**, 895 (1964).
7. Staining solution consisted of a mixture of 10 ml of 0.1*M* tris-HCl buffer, *p*H 8.0; 0.1 ml of 0.1*M* $MgCl_2$; 10.0 ml MTT tetrazolium, 2 mg/ml; 0.5 ml of 6-phosphogluconate, 20 mg/ml; 0.2 ml triphosphopyridine nucleotide, 10 mg/ml; 0.05 ml phenazine methosulfate, 5 mg/ml (Sigma Chemical Co.).
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Aminoaciduria Resulting from Cycloleucine Administration in Man

Abstract. *Cycloleucine (1-aminocyclopentanecarboxylic acid) administration in cancer patients resulted in a reversible marked aminoaciduria consisting predominantly of cystine, ornithine, lysine, and arginine, with only minor elevations of other amino acids. Plasma levels of amino acids were essentially unchanged except for slight decreases in ornithine and lysine. This aminoaciduria is most likely due to a blockage by the drug of the renal tubular reabsorption of cystine, ornithine, lysine, and arginine, resulting in an aminoaciduria of the type seen in cystinuria.*

The nonmetabolizable α-amino acid, 1-aminocyclopentane-1-carboxylic acid (cycloleucine, NSC-1026) has been reported to inhibit growth of several experimental tumors (*1*, *2*). Clinical trials have generally been unimpressive in all patients with the possible exception of those with leiomyosarcoma (*3*). Studies by Sterling and Henderson (*4*) indicated that the drug inhibited incorporation of certain amino acids into proteins of ascites tumors and that the effect was probably due to inhibition of transport of certain amino acids into the tumor cells; however, Berlinguet *et al.* (*5*) presented evidence that cycloleucine inhibited protein synthesis in vivo and in vitro, presumably by interference with the formation of aminoacyl-transfer RNA complexes. Other studies had shown that cycloleucine did not inhibit transamination of amino acids nor did it inhibit their enzymatic oxidation (*6*). The drug was not incorporated into protein and was not further metabolized or degraded in animals (*6*, *7*). Cycloleucine has been used in studies of amino acid transport and has been shown to act competitively in the active transport of several amino acids (*8*). Recently Clark *et al.* (*9*) reported

Table 1. Urinary amino acid levels (micromoles per day) of patients before and during treatment with cycloleucine.

Patient	Cystine		Ornithine		Lysine		Arginine	
	Before	During	Before	During	Before	During	Before	During
E	156	4,748	36	10,150	184	40,845	37	1,720
T	66	5,507	18	2,050	120	8,240	25	2,894
K	158	4,640		1,685	136	8,894		1,458
Z	161	5,200	27	3,520	106	13,100	14	3,500
W	168	5,450	29	2,880	332	5,530	17	1,670
Average (μmole/day)	142	5,109	28	4,057	175	15,322	23	2,248
Average (mg/day)	34	1,228	3.7	536	26	2,240	4.0	392