

Anemia, splenomegaly, and increased osmotic fragility of erythrocytes in Abyssinian and Somali cats

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Objective—To determine clinical and clinicopathologic features of a chronic intermittent severe hemolytic anemia characterized by erythrocyte osmotic fragility in Abyssinian and Somali cats.

Design—Case series.

Animals—13 Abyssinian and 5 Somali cats.

Procedures—History, pedigree information, and results of routine laboratory tests, special erythrocyte studies, and histologic evaluation of splenic and hepatic specimens were analyzed.

Results—Age at which clinical signs of anemia were first apparent ranged from 6 months to 5 years. Ten cats had splenomegaly. Most often, the PCV was between 15 and 25%, but it was as low as 5% at some times. The anemia was characterized by macrocytosis and mild to moderate reticulocytosis, but no poikilocytosis. Hyperglobulinemia, lymphocytosis, mild hyperbilirubinemia, and high hepatic enzyme activities were common findings. Results of Coombs tests and tests for infectious diseases were negative. The erythrocytic osmotic fragility was high in affected cats (mean osmotic fragility, 0.66 to 0.78%), compared with healthy cats (0.48 to 0.58). No specific membrane protein abnormality, erythrocyte enzyme deficiency, or hemoglobinopathy was identified. Histologic evaluation of splenic and hepatic specimens revealed extramedullary hematopoiesis and hemosiderosis. Four of the 5 Somali cats were closely related.

Conclusions and Clinical Relevance—On the basis of results of pedigree analyses, the apparent breed predilection, and the exclusion of other known causes of anemia in cats, we believe that the hemolytic anemia in these cats was likely a result of a novel hereditary erythrocyte defect. A genetic predisposition to immune-mediated destruction of erythrocytes could not be ruled out. (*J Am Vet Med Assoc* 2000;217:1483–1491)

Most anemias in cats are a result of inadequate erythropoiesis or blood loss, but hemolytic anemias have been recognized with increasing frequency. Hemolytic

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anemia may be difficult to diagnose in cats, and there are numerous causes that have to be considered in the differential diagnosis of hemolytic anemia in cats.

Primary or idiopathic (auto-)immune-mediated hemolytic anemia (IMHA), the most common cause of hemolysis in dogs, has only rarely been documented in cats.¹⁻³ However, secondary IMHA has been documented in cats in association with FeLV infection, hemobartonellosis, lymphoma, and feline infectious peritonitis.^{1,4,5} Other immune-mediated disorders such as neonatal isoerythrolysis,^{6,7} acute transfusion reactions secondary to A-B blood type incompatibilities,⁸⁻¹⁰ and drug allergies resulting in hemolysis (eg, an allergic reaction to propylthiouracil)¹¹ have also been described, and hemobartonellosis,¹² cytauxzoonosis (which occurs in the southern United States),¹³ and babesiosis (which occurs in Southern Africa and Southern Europe)¹⁴⁻¹⁶ are infectious causes of hemolytic anemia in cats. In addition, FeLV infection,¹⁷ inflammation,¹⁸ and neoplastic diseases¹⁹ may decrease the lifespan of erythrocytes in cats.

The hemoglobin of cats is more susceptible to oxidation and denaturation than the hemoglobin of other species, because cats have 8 to 10 sulfhydryl groups per hemoglobin molecule, compared with 2 to 4 sulfhydryl groups per hemoglobin molecule in other species. Moreover, the dissociation of hemoglobin tetramers to dimers is 10 times as fast in cats as it is in other species.²⁰ Certain chemical agents and drugs (eg, methylene blue, methionine, phenazopyridine, phenacetin, benzocaine, and acetaminophen) and food components (eg, propylene glycol and onions) have been reported to cause Heinz body hemolytic anemia or methemoglobinemia in cats.²¹⁻²³ Severe hypophosphatemia reportedly can also cause hemolytic anemia in cats.²⁴

Inherited erythrocyte defects, such as enzyme deficiencies, membrane defects, and hemoglobinopathies, are common and well-defined causes of hemolytic anemia in humans and dogs.²⁵ However, hemolytic anemia attributable to inherited erythrocyte defects in cats, such as porphyria²⁶ or pyruvate kinase (PK) deficiency,^{a,b} have only rarely been reported.

The purpose of the study reported here was to determine clinical and clinicopathologic features of a chronic intermittent severe hemolytic anemia characterized by extreme osmotic fragility of erythrocytes, macrocytosis, splenomegaly, and hyperglobulinemia in Abyssinian and Somali cats.

Materials and Methods

Animals—Thirteen Abyssinian (4 male, 9 female) and 5 Somali (3 male, 2 female) cats from throughout the United

States that were examined because of chronic hemolytic anemia were used in the study. In addition, 7 healthy family members of the affected cats and 42 healthy unrelated cats (control cats; 3 Abyssinians and 39 cats of other breeds) were included. Control cats consisted of cats examined at the veterinary teaching hospital at the same time as affected cats and cats in a colony maintained by the University of Pennsylvania School of Veterinary Medicine. Colony cats were cared for according to the *Guidelines for the Care and Use of Laboratory Animals in Research*.

Affected cats ranged from 0.5 to 8 years old at the time of initial examination. Three of these cats were examined at the University of Pennsylvania veterinary teaching hospital, and 4 were examined at the Animal Medical Center in New York City. The remaining 11 cats were examined and treated at a variety of veterinary clinics, but special studies were performed at the veterinary teaching hospital. History, medical records, and pedigree information of affected cats were collected. Follow-up time of affected cats ranged from 1 to 2 years.

Sample collection—Blood samples were anticoagulated with EDTA. Samples were chilled after collection to 4 C and stored or shipped at 4 C. All blood samples were analyzed within 24 hours after collection.

Laboratory testing—Standard clinical laboratory techniques were used to measure PCV and perform CBC, serum biochemical analyses, serum protein electrophoresis, and urinalyses. Reticulocyte counts were performed manually, and reticulocyte fractions were corrected to a PCV of 37%. Blood smears were evaluated for *Haemobartonella felis* and abnormalities of erythrocyte morphology. Serum iron concentration, serum ferritin concentration, and total iron binding capacity were measured²⁷ in 4 affected cats.

Coombs tests (direct antiglobulin test) were performed on all affected cats 1 to 5 times. For 8 cats, Coombs tests were performed or repeated at the veterinary teaching hospital, using a polyvalent IgG antiserum.^c For these cats, tests were performed immediately after collection of blood samples (for cats at the teaching hospital) or as soon as samples were received (typically, after overnight shipment and within 24 hours after sample collection). Erythrocytes were washed carefully 3 times with phosphate-buffered saline solution in preparation for the Coombs test and to evaluate for persistent autoagglutination. Erythrocyte suspensions were incubated with antiglobulin reagent at 37 C for 30 minutes and overnight at 4 C and evaluated for degree of agglutination and hemolysis.

Tests for FeLV antigen were performed in all cats, and tests for feline immunodeficiency virus (FIV) antibodies were performed in 15 cats. The feline infectious peritonitis (FIP) titer was measured in 10 cats, and the toxoplasmosis titer was measured in 2. In 7 cats, a bone marrow aspirate was obtained and submitted for cytologic evaluation. An immunofluorescence assay to detect FeLV antigen was performed on fixed blood or bone marrow smears of 5 affected cats.

Erythrocyte osmotic fragility—The erythrocyte osmotic fragility test measures the stability of erythrocytes in sodium chloride solutions ranging from 0.85 to 0%.²⁸ All samples were tested within 36 hours after blood sample collection. Each time a blood sample from an affected cat was tested, a blood sample from a control cat was tested at the same time. In some affected cats, erythrocyte osmotic fragility was assessed on several occasions.

For measurement of erythrocyte osmotic fragility, aliquots (30 μ l) of each blood sample were suspended in 4 ml of serial dilutions of phosphate-buffered sodium chloride solution (pH 7.4). Solutions were incubated for 30 minutes

at 22 C and centrifuged, and degree of hemolysis in the supernatant was measured photometrically at 540 nm. So that similar numbers of erythrocytes would be used when testing samples from anemic and healthy cats, blood samples were corrected to a PCV of approximately 30% by adding phosphate-buffered saline solution or removing plasma. The degree of hemolysis following addition of erythrocytes to a 0.85% sodium chloride solution without incubation was considered baseline hemolysis. Complete lysis (100%) was achieved by incubating erythrocytes in water. Mean erythrocyte osmotic fragility was determined from the lysis curve as the concentration of sodium chloride at which 50% of the erythrocytes were hemolyzed.

Lysis curves and mean erythrocyte osmotic fragility were also determined for blood samples from the 42 control cats. Results for these cats did not seem to be associated with breed, age, or sex.

Erythrocyte membrane proteins—For 9 affected cats, erythrocyte membrane proteins were examined by means of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Hemoglobin-free erythrocyte membranes (ghosts) were prepared by adding proteolysis inhibitor solution (final concentration: 2 mmol diisopropylfluorophosphate/L, 10 g leupeptin/ml, and 10 g pepstatin/ml^d) to erythrocyte suspensions, which were then exposed to hypotonic solutions.²⁹ Erythrocyte membrane proteins were separated by means of electrophoresis on a 7.5% gel, using the buffer system by Laemmli.³⁰

Transmission electron microscopy—Erythrocytes from 2 affected cats were examined by means of transmission electron microscopy. Cells were fixed in 4% paraformaldehyde and 5% glutaraldehyde and postfixed in 1% osmium tetroxide.

Erythrocyte enzyme activities—Pyruvate kinase activity was measured in 6 cats, as described.³¹ Erythrocytes were purified by means of cellulose column filtration to remove leukocytes. An immunoblot analysis, using polyclonal anti-PK antibody,^{32,33} was performed in 3 affected cats, and DNA-based PK activity testing^b was performed in 10 affected cats. Additional glycolytic and pentose phosphate shunt enzyme activities were measured in erythrocytes from 2 cats.³¹

Hemoglobin analysis—Hemoglobin from 6 affected cats was studied by means of agarose gel isoelectric focusing^e to separate the hemoglobin tetramers HbA and HbB. In addition, α - and β -globins in hemolysates from 8 affected cats were separated by means of reversed-phase high-performance liquid chromatography (RP-HPLC).³⁴

Histologic analyses—Splenic samples from 6 affected cats that underwent splenectomy and 1 splenic, 5 hepatic, and 2 lymph node biopsy specimens were examined histologically. Tissue specimens were fixed in buffered 10% formalin and embedded in paraffin. Thin (5 μ m) sections were cut and stained with H&E. Additional sections from 5 cats were stained with Prussian blue stain for iron and with Fouchet stain for bile.

Evaluation for infectious diseases—Fresh-frozen splenic samples were submitted for bacterial culture, virus isolation, and electron microscopy for identification of viral, bacterial, and parasitic organisms.

Results

Clinical findings—For the 18 affected cats, mean age at which clinical signs of anemia were first apparent was 23 months (range, 6 months to 5 years). Most owners sought veterinary care for their cats because of

lethargy (16), anorexia (14), weight loss (11), and pale mucous membranes (7). Other findings were a palpable spleen (4), icterus (3), vomiting (3), collapsing, shaking, or seizing (3), fever (2), poor coat (1), colitis (1), urinary tract problems (1), tachypnea (1), tachycardia (1), and polyuria and polydipsia (1).

The most common clinical abnormalities on initial examination were lethargy ($n = 16$) and pale mucous membranes (15). Ten cats were determined to have splenomegaly on the basis of results of abdominal palpation, radiography, ultrasonography, or surgery. Splenomegaly appeared to be more prominent in older cats; the 3 cats between 6 and 8 years old had the largest spleens. Other findings were mild icterus (4), fever (2), lymphadenopathy (2), and tremor (1). Six cats had intervals without clinical signs of disease and PCV $> 30\%$. Eleven cats had severe hemolytic episodes (PCV as low as 5%), whereas the other cats did not have any overt hemolytic episodes, and PCV was $> 14\%$.

Treatment and outcome—Various treatments were administered to affected cats. All cats were treated at least once with doxycycline ($n = 15$) or with other antibiotics such as enrofloxacin and amoxicillin (3). Prednisolone was administered at immunosuppressive dosages (1 to 2 mg/kg [0.45 to 0.9 mg/lb] of body weight, PO, q 12 h) to all cats. The dosage was usually tapered when clinical improvement and an increase in PCV were noticed. The effect of prednisolone treatment was difficult to judge, but at least 7 cats did not appear to improve during treatment with prednisolone, and other cats appeared to improve without prednisolone treatment. Two cats received cyclophosphamide in addition to prednisolone. One or more blood transfusions were necessary in 6 cats during hemolytic episodes (4) or before splenectomy (4).

Splenectomy was performed in 6 cats that did not respond to treatment or had recurrent hemolytic episodes. Age at the time of surgery ranged from 2 to 9 years (mean, 5 years). In all 6 cats, the spleen was several times normal size; spleens ranged from 45 to 196 g (reference range, 5 to 32 g; Fig 1). The PCV seemed



Figure 1—Intraoperative appearance of the spleen of a 2.9-kg (6.4-lb) Abyssinian cat with increased osmotic fragility of erythrocytes. The spleen constituted 6% of the cat's body weight (reference range, $< 1\%$).



Figure 2—Appearance of EDTA-anticoagulated blood samples from an Abyssinian cat with increased osmotic fragility of erythrocytes (left) and from a healthy cat (right) following 1 day of storage in the refrigerator. Notice the extensive hemolysis of the sample from the affected cat.

to stabilize after splenectomy, and weight gain was reported. Two of the splenectomized cats were alive 3 and 18 months after surgery, whereas the other 4 cats were euthanatized between 8 and 16 months after surgery because of recurrent anemia.

The remaining 12 cats did not undergo splenectomy. Six of these cats died or were euthanatized during hemolytic episodes 2 and 3 months and 2, 2.5, 3, and 7 years after the onset of anemia; cats were between 1 and 8 years old (mean, 4.2 years) at the time of death. Five cats were still alive at follow-up times ranging from 1.5 to 2 years. One cat was still alive when it was lost to follow-up 3 months after initial examination.

Laboratory testing—Most blood samples were extremely hemolyzed after 1 day of refrigeration or overnight shipping on ice to the veterinary teaching hospital (Fig 2). Because of the extreme *in vitro* fragility of the erythrocytes, some of the routine laboratory tests, as well as several of the special erythrocyte studies, were hampered. The PCV ranged from 5 to 42% but was most commonly between 15 and 25% (Fig 3). The PCV at the time of initial examination was 6 to 28% (mean, 18%). During the course of the study, the PCV was $> 30\%$ at least once in 6 cats. If macroscopic agglutination or hemoglobinemia was evident, CBC were not performed. **Mean corpuscular volume (MCV)** of erythrocytes from affected cats ranged from 45 to 102 fl (mean \pm SD, 67 ± 16 fl; Table 1), but 75% of the time, MCV was between 52 and 85 fl. Mean corpuscular hemoglobin concentration ranged from 28 to 35 g/dl.

The reticulocyte fraction ranged from 0.1 to 28.5% (corrected reticulocyte fraction, 0.1 to 24.5%; absolute reticulocyte count, 4,100 to 454,000/ l). In most cats, the anemia was mildly (corrected reticulocyte fraction, 0.5 to 1.5%; absolute reticulocyte count, 20,000 to 95,000/ l; $n = 7$) or moderately (corrected reticulocyte fraction, 1.5 to 4%; absolute reticulocyte count, 110,000 to 195,000/ l; 7) regenerative, whereas 2 cats had a strong regenerative response (corrected reticulocyte fraction, $> 4\%$; absolute reticulocyte count, $> 250,000/ l$), and 2 cats had nonregenerative anemia

Table 1—Results of hematologic and serum biochemical tests for 18 Abyssinian and Somali cats with chronic hemolytic anemia associated with increased erythrocyte osmotic fragility

Variable	Affected cats		Reference range
	Mean	Range	
Hematocrit (%)	18.7	5–42	30–45
Hemoglobin (g/dl)	5.6	2.5–12	9–15
RBC ($\times 10^9/\mu\text{l}$)	3.1	0.6–10	6–12
MCV (fl)	67	45–102	38–49
MCHC (g/dl)	31.2	28–35	30–35
MCH (pg)	14.3	12–19	13–18
Reticulocytes			
Corrected fraction (%)	2.3	0.1–24.5	< 0.4
Absolute count ($\times 10^3/\mu\text{l}$)	135	4.1–454	< 40
Nucleated RBC (/100 WBC)	13.3	0–73	< 1
WBC count ($\times 10^3/\mu\text{l}$)	12.1	3.6–48	6–17
Neutrophils ($\times 10^3/\mu\text{l}$)	5.5	0.3–9.9	2.5–12.5
Lymphocytes ($\times 10^3/\mu\text{l}$)	4.5	0.2–11.8	1.5–5.0
Monocytes ($\times 10^3/\mu\text{l}$)	0.4	0–2.2	0–0.9
Eosinophils ($\times 10^3/\mu\text{l}$)	0.3	0–1.8	0–1
Basophils ($\times 10^3/\mu\text{l}$)	0.1	0–0.5	0
Bilirubin (mg/dl)	0.8	0.1–2.9	0.1–0.4
Total protein (g/dl)	8.8	6.4–11.8	5.5–7.7
Globulin (g/dl)	5.7	3.2–9.1	2.1–4.0
Phosphorus (mg/dl)	4.8	3.2–7.3	3–8
Alanine transaminase (U/L)	123	35–420	< 70
Aspartate transaminase (U/L)	90	40–287	< 100
Alkaline phosphatase (U/L)	46	8–94	< 50
Lactate dehydrogenase* (U/L)	556	31–990	63–193
Creatine kinase† (U/L)	1,663	90–7,730	< 200
Iron‡ ($\mu\text{g/dl}$)	180	108–259	33–135
Total iron binding capacity‡ ($\mu\text{g/dl}$)	824	428–980	169–325
Ferritin‡ (ng/ml)	1,844	112–3,875	32–12

*Only 5 affected cats were tested. †Only 7 affected cats were tested. ‡Only 4 affected cats were tested.

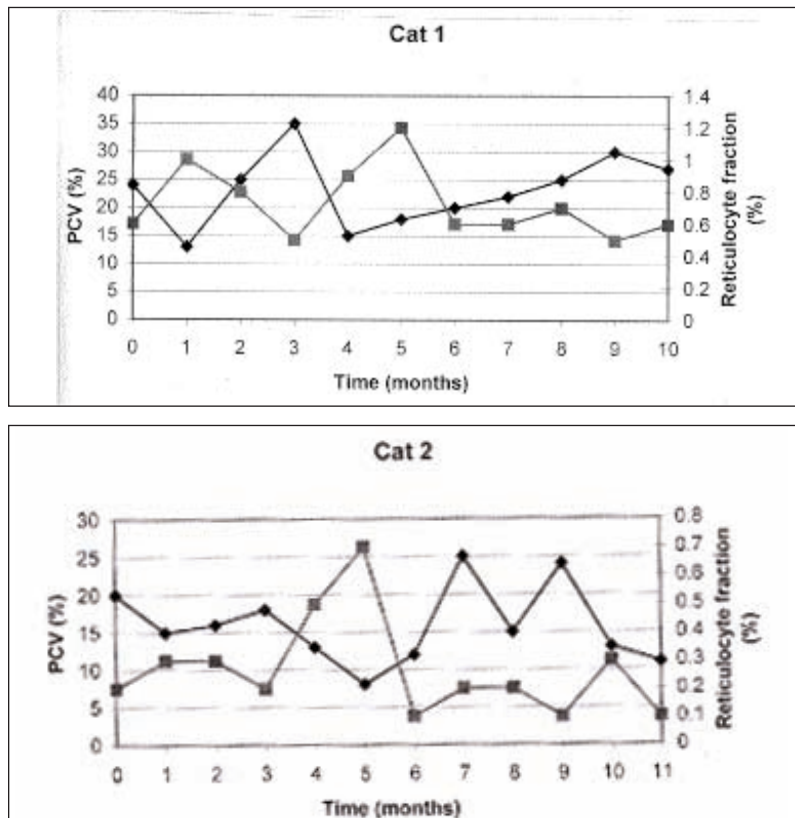


Figure 3—Representative time courses of the PCV (◆) and corrected reticulocyte fraction (■) in a 3-year-old spayed female Somali cat (cat 1) and an 8-year-old castrated male Abyssinian cat (cat 2) with chronic hemolytic anemia associated with increased erythrocyte osmotic fragility.

(corrected reticulocyte fraction, < 0.5%; absolute reticulocyte count, < 20,000/ l). The 2 cats with nonregenerative anemia were 8 years old and were the oldest affected cats.

In 11 cats, WBC counts were in the reference range (6 to $18 \times 10^3/ \text{l}$) during the course of the disease. Six cats had leukocytosis (WBC count $> 18 \times 10^3/ \text{l}$) at the time of initial examination. Marked leukocytosis (WBC count $> 24 \times 10^3/ \text{l}$) developed in 3 of these cats after splenectomy. Ten cats had lymphocytosis; 5 cats had lymphocyte counts $> 7,500/ \text{l}$. Reactive lymphocytes were detected in 5 cats. One cat had intermittent leukopenia (WBC count $< 3,200/ \text{l}$).

Examination of blood smears revealed anisocytosis, polychromasia, and macrocytosis. No poikilocytes were observed except for rare stomatocytes (Fig 4). Howell-Jolly bodies and macroscopic agglutination were detected in blood smears from 9 cats. In all cats, the agglutination broke up after 3 washings of EDTA-anticoagulated blood with buffered saline solution. Slight hypochromasia or rouleaux was detected in 6 cats. No *Haemobartonella felis* organisms were detected, and platelet morphology appeared normal.

Serum total bilirubin concentration was normal or slightly high during the course of the disease. Serum hepatic enzyme activities were high in 9 cats. Hyperglobulinemia with serum globulin concentration ranging from 4.3 to 9.1 g/dl was detected in 12 cats, and serum protein electrophoresis indicated a polyclonal gammopathy in all 5 cats tested. Bilirubinuria and hemoglobinuria were detected in 4 of 7 cats tested.

Results of FIV antibody and FeLV antigen tests, as well as immunofluorescence assays to detect FeLV antigen on fixed blood or bone marrow smears, were negative. All 10 cats tested were seronegative for FIP or had titers $< 1:200$. Results of Coombs tests performed with a polyvalent reagent at 37 C and 4 C were negative for 13 cats. For 3 of these cats, results of a Coombs test performed at an outside laboratory were positive, but results of a follow-up test performed at the veterinary teaching hospital were negative. A Coombs test could not be performed on the other 5 cats because of severe hemolysis or agglutination of erythrocytes after shipping.

Cytologic examination of bone marrow aspirates from 6 cats revealed mild

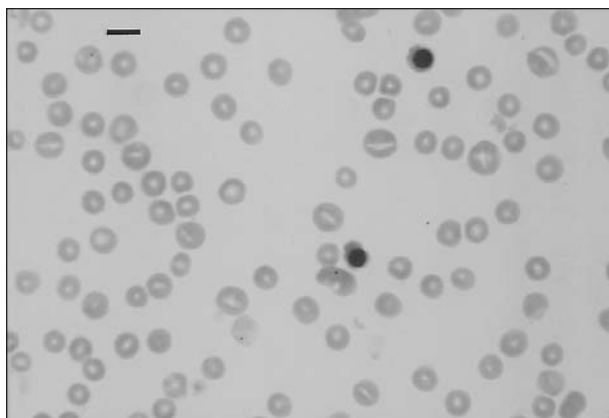


Figure 4—Photomicrograph of a blood smear from a female Abyssinian cat with chronic hemolytic anemia associated with increased erythrocyte osmotic fragility. Notice the anisocytosis, polychromasia, a few stomatocytes, and 2 nucleated RBC. Wright stain; bar = 10 μ m.

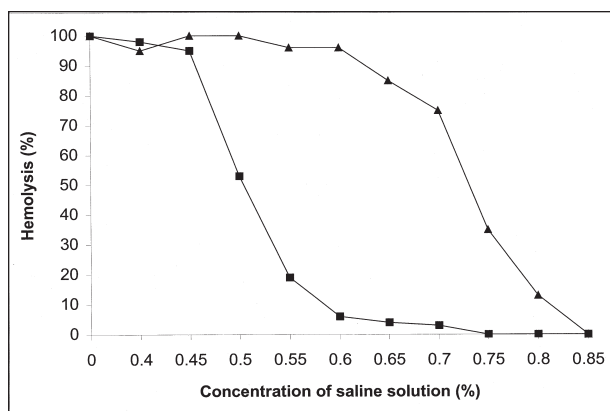


Figure 5—Erythrocyte osmotic fragility curve for a 2-year-old female Somali cat with chronic hemolytic anemia (▲; mean osmotic fragility, 0.73%) and for a healthy control cat (■; mean osmotic fragility, 0.51%).

to moderate erythroid hyperplasia, with normal myeloid and megakaryocytic distribution. An 8-year-old cat with nonregenerative anemia had erythroid hyperplasia.

A severely enlarged spleen with homogeneous slight hyperechogenicity was observed in the 3 cats that underwent abdominal ultrasonography.

Erythrocyte osmotic fragility—Erythrocyte osmotic fragility was measured in all cats 2 to 32 hours after blood samples were collected. In 3 cats, erythrocyte osmotic fragility was measured up to 5 times over an 18-month period. The lysis curve for affected cats was markedly shifted toward higher saline solution concentrations, compared with the lysis curve for control cats (Fig 5). The lysis curves for erythrocytes from control and affected cats did not overlap, indicating that the entire erythrocyte population from affected cats was more fragile than normal. Mean erythrocyte osmotic fragility for the 42 control cats ranged from 0.48 to 0.58% (mean \pm SD, 0.54 \pm 0.03%). Mean erythrocyte osmotic fragility for the affected cats ranged from 0.66 to 0.78% (0.75 \pm 0.03%). Baseline hemolysis

of samples from affected cats ranged from 4 to 90% (26 \pm 27.5%), whereas baseline hemolysis for control cats ranged from 0 to 3%.

Even during periods when cats were clinically normal, erythrocyte osmotic fragility was high. In 5 affected cats, mean osmotic fragility ranged from 0.66 to 0.77% when PCV was between 32 and 42%. Two cats with mean osmotic fragility of 0.75 and 0.76% prior to undergoing splenectomy had mean osmotic fragility of 0.57 and 0.55%, respectively, 1 month after surgery. Mean osmotic fragility was measured in 1 of these cats 10 months later and was again high (0.68%).

Erythrocyte membrane proteins—Analysis of erythrocyte membranes by SDS-PAGE did not reveal any obvious deficiency in any membrane protein band in affected, compared with control, cats (Fig 6). Four cats had a prominent band 4.5 (45,000 kD), but bands 1, 2, 3, 4.1, and 4.2 were less prominent, probably because of degradation artifacts when ghosts of these fragile erythrocytes were prepared. After addition of proteinase inhibitors during the membrane preparation step, the intensity of band 4.5 decreased.

Transmission electron microscopy—Transmission electron microscopic examination of erythrocytes from 2 affected cats did not reveal any obvious abnormalities.

Erythrocyte enzyme activities—Erythrocyte PK activity measured in 6 cats ranged from 105 to 167%, compared with activity in control cats (mean \pm SD, 100 \pm 26%). The modest increase in erythrocyte PK activity in affected cats was likely a result of younger erythrocyte populations. One cat had a PK activity of only 60%, carried 1 mutant PK allele, and was, therefore, considered to be a carrier for PK deficiency.⁴ Immunoblot analysis with polyclonal anti-PK antibody in 3 affected cats indicated that the adult erythrocyte isozyme form of PK (R-PK) was present; DNA analysis in 10 affected cats did not reveal any PK mutations. Finally, analysis of the activities of other glycolytic and pentose phosphate shunt enzymes³¹ in erythrocytes from 2 affected cats did not reveal any deficiencies.

Hemoglobin analysis—In 6 affected cats, separation of hemoglobin tetramers by isoelectric focusing revealed 2 hemoglobin bands of different intensities. For 8 affected cats, the α - and β -globin patterns, determined by means of RP-HPLC, were similar to published patterns for healthy cats and cats with anemia of various causes.³⁴

Pedigree analysis—Pedigrees from 5 affected Abyssinian cats were available, but a common ancestor could not be identified. Anecdotally, breeders and veterinarians were aware of related cats that had anemia, but the cause of the anemia in these cats remained undefined. Four of the affected Somalis were closely related but had been raised separately in various parts of the United States (Fig 7). Four parents or siblings of affected Somalis were available for testing; mean erythrocyte osmotic fragility for these 4 cats was normal (mean, 0.56%; range, 0.51 to 0.60%). Of 8 related Somali cats (4 of them shown in Fig 7), 6 were recog-

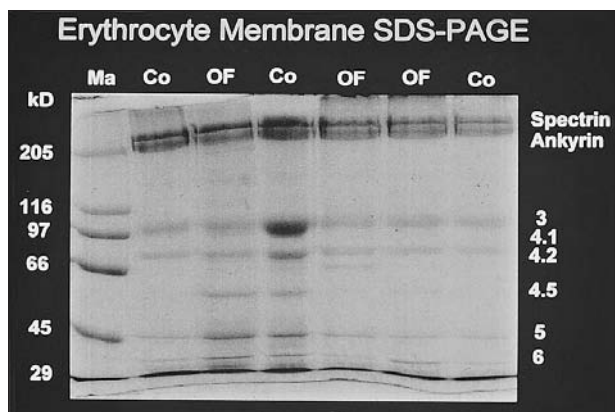


Figure 6—Sodium dodecyl sulphate-polyacrylamide gel electrophoretogram of erythrocyte membranes of 3 cats with chronic hemolytic anemia associated with increased erythrocyte osmotic fragility (OF) and 3 healthy control cats (Co). Lane Ma = Size markers. Numbers on the left side represent molecular sizes; numbers on the right side represent the protein bands.

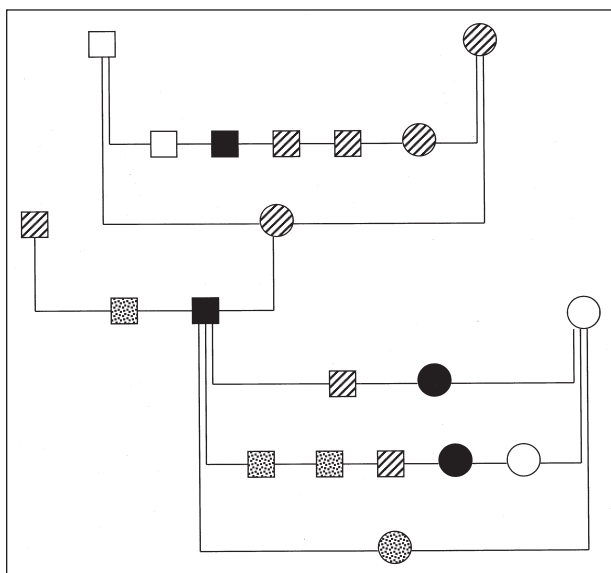


Figure 7—Pedigree analysis of 4 Somali cats with chronic hemolytic anemia associated with increased erythrocyte osmotic fragility: ■ = Affected cats. □ = Healthy cats with normal erythrocyte osmotic fragility. ▨ = Cats that died of anemia but for which erythrocyte osmotic fragility was not tested. ▩ = Cats not available for testing. Squares = Males. Circles = Females.

nized to be anemic between 1 and 4 years of age (mean 2.2), and 2 died at an early age (4 and 6 months) of unknown cause.

Histologic analyses—Histologic evaluation of the spleen from 7 cats ranging from 1 to 9 years old (mean, 4.4 years) revealed marked extramedullary hematopoiesis, hemosiderosis, lymphoid hyperplasia, and diffuse congestion of the red pulp. In addition, mild to marked hepatic extramedullary hematopoiesis and hemosiderosis were found (Fig 8). Extensive iron storage in Kupffer cells and hepatocytes was documented by examination of sections stained with Prussian blue stain, but examination of sections stained with special stains for bile did not reveal accu-

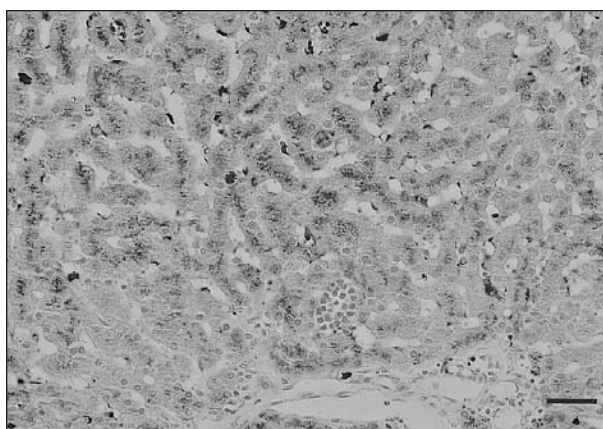
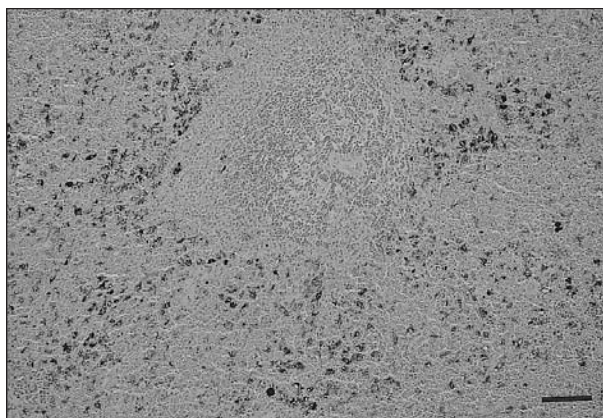


Figure 8—Photomicrographs of sections of spleen (top) and liver (bottom) from an Abyssinian cat with chronic hemolytic anemia associated with increased erythrocyte osmotic fragility. Notice the extensive iron deposition within splenic macrophages and the accumulation of iron material within hepatocytes and Kupffer cells. Perl stain; bars = 90 (top) and 45 (bottom) μ m.

mulation of bile. The two 8-year-old cats had multifocal nodular hepatic regeneration or moderate hepatocellular vacuolization with pigmentation. Examination of lymph node biopsy samples from the 2 cats with lymphadenopathy revealed reactive hyperplasia compatible with antigenic stimulation.

Discussion

The 18 Abyssinian and Somali cats in the present study all had a chronic intermittent hemolytic anemia that was associated with splenomegaly in 10 of the 18. The diagnosis of hemolytic anemia was made on the basis of the finding of regenerative anemia, fragile erythrocytes, and hepatic and splenic hemosiderosis. On the basis of the results of pedigree analyses, the apparent breed predilection, and the exclusion of other known causes of anemia in cats, we believe that the hemolytic anemia in these cats was likely a result of a novel hereditary erythrocyte defect. We did not determine the nature of the defect in these cats, but a membrane defect seems most likely.

All affected cats in the present study had a severe increase in osmotic fragility of their erythrocytes. The osmotic fragility assay is a rough index of erythrocyte surface-to-volume ratio. When erythrocytes are placed in a hypotonic solution, water is drawn osmotically

into them, resulting in cell swelling. When the critical lytic volume is reached, the erythrocyte membrane first leaks and then bursts. Any change in the erythrocyte membrane (eg, membrane skeleton defects or antibody and complement binding) allows hemoglobin to escape directly without osmotic swelling.³⁵ Increases in osmotic fragility are correlated with decreases in survival time of erythrocytes.³⁶ Interestingly, erythrocytes from affected cats in the present study had such high osmotic fragility that they started to lyse in vitro even when stored in a refrigerator for only 1 day. Furthermore, even when the PCV of affected cats was within reference limits, osmotic fragility was high, suggesting that cats had an underlying erythrocyte membrane defect. Splenectomy resulted in a transient improvement in osmotic fragility in some cats, suggesting that the spleen was affecting erythrocyte membrane stability. Further membrane studies, however, remained inconclusive, and the precise membrane defect was not determined.

Increased osmotic fragility in humans with hereditary erythrocyte defects, such as hereditary spherocytosis, elliptocytosis, and stomatocytosis and RH deficiency syndrome, has been described.³⁷ In dogs, inherited erythrocyte defects causing fragile erythrocytes include stomatocytosis^{38,39,f} and spherocytosis.⁸ Protein and molecular characterization of the erythrocyte membrane has been accomplished in human patients with inherited diseases such as spherocytosis and elliptocytosis.³⁷ A band-4.1 deficiency was identified in a mixed-breed dog with elliptocytosis,⁴⁰ and a partial spectrin deficiency was found in Golden Retrievers with hereditary spherocytosis.⁵ Because of the stickiness of the erythrocyte membranes from affected cats in the present study, preparation of ghosts and SDS-PAGE of membrane proteins was technically difficult; however, a lack or deficiency of one of the protein bands in anemic cats, compared with control cats, was not obvious.

In people with hereditary stomatocytosis and increased osmotic fragility, high erythrocyte cation and water contents have been found.³⁷ Hereditary stomatocytosis in dogs has been associated with high erythrocyte sodium and water contents³⁸ or with aberrations in the lipid composition of the erythrocyte membrane.⁴¹ Lipid composition and cation content of erythrocytes from affected cats in the present study were not determined.

Affected cats in the present study had persistent macrocytosis, even when the PCV was within reference limits, and agglutination and reticulocytosis were not evident. Absolute reticulocyte counts did not correlate with size of the erythrocytes, and erythrocytes appeared large on blood smears, compared with other cells.

Extremely high MCV were presumably a result of agglutination of erythrocytes in the cell counting fluid. Macrocytosis in people and dogs with hereditary stomatocytosis has been associated with an increase in intracellular water content.^{37,38} Cats in the present study had few stomatocytes, which is remarkable, as feline erythrocytes are normally small and appear spherical on blood smears. Macrocytosis and

nonregenerative anemia have been identified in cats infected with FeLV⁴² and in cats with myeloproliferative disorders⁴³ and dietary folate deficiency,^h but none of the cats in the present study had any of these conditions.

Immune-mediated hemolytic anemia and hemobartonellosis can markedly increase erythrocyte osmotic fragility in cats⁴⁴; therefore, the possibility that anemia in these cats was attributable to 1 of these disorders had to be carefully excluded. Because there were no reliable diagnostic tests for *Haemobartonella felis* infection available, most cats were empirically treated with doxycycline once or several times during episodes of anemia. In addition, *Haemobartonella* organisms were not identified during cytologic examination of fresh blood smears. The age of the cats at the onset of disease, as well as the fact that Abyssinians and Somalis are related breeds, suggests that affected cats had an inherited problem. It has been suggested that humans and dogs may have a genetic predisposition toward familial IMHA.^{45,46} In the cats of the present report, however, results of Coombs tests performed at the veterinary teaching hospital were consistently negative, and agglutination did not persist after erythrocytes were washed with saline solution. Thus, there was no evidence of an antibody-mediated process. Moreover, at least 7 cats did not respond to immunosuppressive treatment. Nevertheless, IMHA cannot be completely ruled out.

Another potential cause of hemolytic anemia in the Abyssinians and Somalis described in the present report that had to be ruled out was PK deficiency, as this disease has been found in these 2 breeds.^{a,b} Cats with PK deficiency share several clinical and hematologic features with affected cats in the present study such as young age of onset, chronicity of hemolysis, hyperglobulinemia, and splenomegaly. In PK-deficient cats, however, osmotic fragility is normal or only slightly increased.^b In addition, cats in the present study lacked the known PK mutation,^b and erythrocyte PK activity was high. Other erythroenzymopathies were also excluded, and the hemoglobin pattern in affected cats was similar to the pattern in control cats, making a hemoglobinopathy unlikely.

In some cats in the present study, the anemia waxed and waned. The reason for intermittent hemolytic episodes was not clear, but acute exacerbations of the disease may have been a result of stress-inducing events that caused nonspecific activation of the macrophage system, exaggerated damage of erythrocyte membranes, and hematopoietic suppression. Two cats had a history of vaccination or colitis a few days prior to an episode of anemia. Similarly, PK deficiency in cats^b and phosphofructokinase deficiency⁴⁷ in dogs cause intermittent hemolytic episodes.

The mild hyperbilirubinemia and regenerative anemia in these cats were suggestive of a hemolytic process; however, serum and urine bilirubin concentrations were normal in several cats. This may suggest adequate hepatic clearance of heme metabolites by the macrophage and hepatic systems or low grade hemolysis with adequate clearance.

Most affected cats in the present study had poly-

clonal hyperglobulinemia and lymphocytosis, suggesting that they had chronic immune stimulation of unknown origin. Similar findings have been reported for cats with other hereditary erythrocyte defects.²⁵ Several diseases causing hyperglobulinemia in cats such as chronic bacterial, fungal, or parasitic infections, FIP, chronic inflammatory disease, and neoplasia were excluded.

Splenomegaly is a common feature in humans and dogs with chronic hemolytic disease secondary to erythrocyte membrane or enzyme defects^{25,37} and has also been observed in cats with PK deficiency.^{a,b} Splenic enlargement in cats in the present study, however, appeared to be more severe and was caused by congestion of the red pulp, marked extramedullary hematopoiesis, lymphoid hyperplasia, and hemosiderosis. Hemosiderosis was accompanied by high serum iron and ferritin concentrations and total iron binding capacity. Severe hemosiderin deposition within Kupffer cells and hepatocytes could be explained by chronic hemolysis. Human patients with chronic hemolytic anemia, particularly those that receive multiple transfusions, develop hemosiderosis, although hemosiderosis commonly occurs independently of hemolysis in people.

All cats in the present study were initially treated with prednisolone, because an immune-mediated disease was suspected. A transient or partial response was found in some cats and presumably was a result of glucocorticoid-induced impairment of erythrophagocytosis in the spleen and membrane stabilization. The lack of response in many cats suggested that anemia was not a result of an immune-mediated mechanism.

Splenectomy was performed in 6 cats that did not respond to treatment or had recurrent hemolytic episodes. Four cats had a favorable response, with stabilization of the PCV and weight gain after splenectomy. Because bacterial sepsis and thromboembolic complications are potential risks of splenectomy,⁴⁸ this procedure should be reserved for animals with severe splenomegaly associated with erythrocyte destruction, inappetence, and weight loss.

Pedigree analysis revealed that 4 of the Somali cats in the present study were closely related, and 4 relatives had died of anemia, strongly suggesting a genetic basis for the disease. Because both sexes were involved and parents appeared unaffected, an autosomal recessive mode of inheritance appears likely. Further studies, however, are needed to determine the molecular basis and inheritance of this disease.

^aFord S, Giger U, Duesberg C, et al. Inherited erythrocyte pyruvate kinase (PK) deficiency causing hemolytic anemia in an Abyssinian cat (abstr). *J Vet Intern Med* 1992;6:123.

^bGiger U, Rajpurohit Y, Skelly B, et al. Erythrocyte pyruvate kinase deficiency in cats (abstr), in *Proceedings*. First Int Genet Dis Conf, 1998.

^cOrganon Teknika Corp, Durham, NC.

^dSigma Chemical Co, St Louis, Mo.

^eHb Testkit, Isolab, Akron, Ohio.

^fGiger U, Amador A, Meyers-Wallen V, et al. Stomatocytosis in Miniature Schnauzers (abstr), in *Proceedings*. Annu Meet Vet Med Forum 1988;754.

^gSlappendel RJ. Hereditary spherocytosis associated with spectrin deficiency in Golden Retrievers (abstr), in *Proceedings*. European Soc Vet Intern Med, 1998;131.

^hMyers S, Giger U. Macrocytic anemia caused by naturally-occurring folate deficiency in the cat (abstr). *Vet Pathol* 1995;32:547.

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