Feline Congenital Erythropoietic Porphyria Associated With Severe Anemia and Renal Disease

Clinical, Morphologic, and Biochemical Studies

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A feline erythropoietic porphyria was studied in an affected female Siamese cat and 2 male offspring. The principal elevated porphyrins were Type I isomers of uroporphyrin and coproporphyrin; the porphyrin precursors, porphobilinogen and δ-aminolevulinic acid, were also detected. Porphyrins were present in the blood and in all the viscera, teeth, bones, and excreta. There was severe macrocytic hypochromic anemia, hepatomegaly, splenomegaly, and uremia associated with a renal disease characterized by mesangial hypercellularity and proliferation (resulting in narrowing of glomerular capillaries) and ischemic tubular injury. There was thickening of tubular basement membranes and tubular epithelial lipidosis, degeneration, and necrosis. Electron microscopic studies of bone marrow and kidney revealed the presence of membrane-enclosed lamellar bodies 150 to 1000 nm in diameter in cytoplasmic and extracellular locations. (Am J Pathol 80:367–386, 1975)

THE PORPHYRIAS are a group of inherited diseases that involve the biosynthetic heme pathway. They are characterized by excessive amounts and abnormal types of porphyrins in tissues, blood, and excreta. The porphyrias of man, which include both congenital and acquired forms, are grouped into two major categories, erythropoietic and hepatic, based on the tissue in which the metabolic defect is expressed. Within each category there are several types, classified according to genetic characteristics, clinical signs, and patterns of heme precursor accumulation. The normal precursors of heme are of the Type III isomer series. Unique to congenital erythropoietic porphyria, Type I isomer porphyrins occur in very high levels.[1-3]

Congenital erythropoietic porphyrias of domestic animals have been described in cattle,[4-7] swine,[8] and cats.[9-13] Detailed studies of feline porphyria are limited to a single case in a cat [9] and its offspring.[10-13] The

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principal porphyrins present were Type I isomers; porphobilinogen was also detected in these cats.

In this paper we describe studies of a family of Siamese cats with an apparently new form of porphyria having some characteristics not previously reported in animals or man. Affected cats had severe anemia, hepatomegaly, splenomegaly, and a peculiar renal disease characterized by marked mesangial hypercellularity, tubular degeneration, and thickening of tubular basement membranes. The major metabolic abnormality was an unusually high level of uroporphyrin I in tissues and excreta.

Materials and Methods

Source of Cats

The index cat (Cat A) was a 10-month-old Siamese female submitted for necropsy about 24 hours after it died following a routine ovariohysterectomy with ether anesthesia. A clinical diagnosis of porphyria made by the attending veterinarians was based on a finding of erythrodontia. The diagnosis was confirmed by necropsy in our laboratories. Two porphyric male offspring (Cats B and C) of the index cat were then located and studied. An attempt was made to establish a breeding colony with Cat B, but it had no libido and became progressively weaker; when moribund at about 27 months of age, it was sacrificed with sodium pentobarbital. Only limited study was permitted on Cat C by its owner. Although anemic and lacking libido, the cat did not seem to be severely affected. It was unexpectedly found dead when it was about 32 months old; necropsy was performed about 18 hours postmortem. For control purposes, 2 normal, unrelated adult cats were sacrificed with sodium pentobarbital and studied.

Histopathology

Tissues were fixed in 10% neutral buffered formalin, dehydrated in ethanol, cleared in xylene, and embedded in paraffin for routine histopathologic examination. Hematoxylin and eosin stain was used for general observation. Periodic acid–Schiff, Gomori's trichrome, Wilder's reticulum, Prussian blue, and Lillie's allochrome stains were employed on paraffin sections. Oil red O stains were used for frozen sections, all according to standard methods.[14]

Fluorescence Microscopy

For immunofluorescent studies, pieces of kidney from Cat B and the 2 control cats were removed immediately after sacrifice, snap-frozen in isopentane cooled with dry ice, and stored at −20 C. Sections were cut at 7-μ thickness on a cryostat microtome, fixed in acetone for 10 minutes, and air-dried.

Sections were stained by the indirect fluorescent antibody method with a 1:20 dilution of rabbit antiserum against feline globulin (Difco Laboratories, Detroit, Mich.), incubated for 30 minutes at 37 C, washed three times for 5 minutes each in phosphate-buffered saline, and air-dried. They were then stained secondarily with a 1:20 dilution of fluorescein-labeled goat antirabbit γ-globulin (Antibodies Inc., Davis, Calif.) for 30 minutes at 37 C, washed three times in phosphate-buffered saline, air-dried, coverslipped with nonfluorescent mounting medium (Difco Laboratories), and examined with a Zeiss photomicroscope with Osram ABO 200 mercury bulb, Zeiss FITC interference filter and barrier filters 43 and 50 (Carl Zeiss, Oberkochen, Germany). Additional controls consisted

of sections stained with a 1:20 dilution of normal rabbit serum followed by the above secondary stain.

For autofluorescent studies of porphyrins in tissues, unfixed frozen sections and formalin-fixed, paraffin-embedded sections were examined. The paraffin-embedded sections included some stained with routine stains and unstained sections which had been deparaffinized with xylene and coverslipped with nonfluorescent mounting medium (Difco Laboratories). Sections were examined with the Zeiss photomicroscope and Osram mercury bulb using Zeiss exciter filters and barrier filters in various combinations.

Electron Microscopy

Selected tissues for electron microscopy were removed from Cat B after sacrifice with sodium pentobarbital. Some formalin-fixed tissues from Cats A and C were also processed for electron microscopy. Tissues were immersed immediately in cold 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer.[15] They were trimmed into 1-mm cubes, fixed for 4 hours at 5 C, and transferred to 0.1 M sucrose-cacodylate buffer overnight. Tissues were postfixed for 1 hour in 2% osmium tetroxide in S-collidine buffer, dehydrated in graded alcohols and propylene oxide, and embedded in Epon 812. Sections 1 μ thick were stained with toluidine blue [16] and PAS. Thin sections were cut with glass and diamond knives on a Porter-Blum ultramicrotome (Model MT-1, Ivan Sorvall, Newton, Conn.), stained with saturated aqueous uranyl acetate and lead citrate,[17] and examined with RCA (Model EMU-2E, RCA, Camden, N.J.), and AEI (Model 6B, Associated Electrical Industries, Ltd., Harlow, England) electron microscopes.

Porphyrin Analyses

All tissues for prophyrin assay were frozen at −20 C until analysis was performed.

Urine

The urine was slightly acidified with 2% hydrochloric acid. Uroporphyrins and coproporphyrins were extracted with *n*-butanol and spotted on a silica gel plate; chromatography was performed. The separated porphyrin spots were identified under ultraviolet light, scraped from the plate, and the porphyrins measured fluorometrically.[18]

Bone

Bone was crushed and cut into small pieces. For each gram of bone, 4 ml of 6 N potassium hydroxide containing 1% EDTA was added. The mixture was stirred for 1 hour, and the KOH solution was decanted and replaced with the same volume of 6 N hydrochloric acid. This was again stirred for 1 hour, then stored overnight in the refrigerator. Essentially complete solubilization of the bone occurred. The KOH and HCl solutions were combined, adjusted to pH 3, and diluted to 180 ml (30 ml/g bone) with distilled water. For porphyrin assay, 1 ml of the solubilized bone was added to 25 ml distilled water. Coproporphyrins and protoporphyrins were extracted with ether. The ether was washed once with 10% sodium acetate and once with water. These washes were added to the uroporphyrin solution (aqueous phase). Coproporphyrin was removed by extracting the ether with 0.1 N hydrochloric acid, and protoporphyrin was removed with 1.5 N hydrochloric acid. The uroporphyrin solution (combined aqueous phases) was adjusted to pH 3.2 and diluted to 100 ml with distilled water. A 20-ml aliquot was taken, from which the uroporphyrin was extracted into *n*-butanol. The *n*-butanol was washed with a few milliliters of water, and the uroporphyrin was removed with 1.5 N HCl. The concentrations of porphyrins in the acid solutions were determined fluorometrically.[18]

Blood and Other Tissues

Blood, kidney, spleen, and liver were made into 5% homogenates with 0.9% sodium chloride. The porphyrins from appropriate quantities of each tissue were extracted using

ten volumes of acetone containing 2% concentrated hydrochloric acid. An equal volume of ethyl acetate was added to separate the phases and remove coproporphyrins and protoporphyrins. After washing with sodium acetate and water to remove uroporphyrin, the coproporphyrin was extracted from ethyl acetate with 0.1 N hydrochloric acid, and the protoporphyrin was extracted with 1.5 N hydrochloric acid. The uroporphyrin solution (combined aqueous phases) was adjusted to pH 3.2, then taken into *n*-butanol. Following one wash with water, the uroporphyrin was extracted with 1.5 N hydrochloric acid. The porphyrin concentrations in all of the acid extracts were measured fluorometrically.[18]

Isomer Analysis

The isomer composition of urinary coproporphyrin was determined using the method of Yuan and Russell.[19]

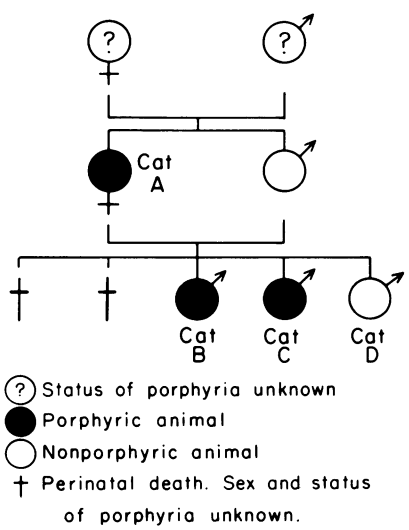
Results

Pattern of Inheritance

The inheritance pattern of this feline porphyria is outlined in Text-figure 1. The status of the original male and female and their other offspring is not known except that they were Siamese cats. The first female known to be affected (Cat A) was born in the autumn of 1969. She mated with her unaffected brother and produced a litter of 5 kittens in the summer of 1970. Two of these died perinatally; the status of porphyria was undetermined. The 3 survivors were males, and 2 of these (Cats B and C) were affected. The third (Cat D) was clinically normal. Each of the 3 affected cats was raised in a different household.

Clinical and Hematologic Features

The affected cats had teeth of an orange-brown color extending from the gumline to the tips, where the discoloration was slightly diminished



TEXT-FIGURE 1—Inheritance pattern of feline congenital porphyria.

(Figure 1). The teeth fluoresced orange-pink under long wavelength ultraviolet light in a dark room. In general, the cats were anemic and appeared depressed and listless, although this varied considerably and seemed to correspond with the severity of anemia. Their hair coats were somewhat roughened and dull. Although no controlled observations were made, the owners reported periodic episodes of nasal and ocular discharge during the summer months. In Cat C, this was diagnosed by a veterinary practitioner as upper respiratory infection and was treated without success.

The urine was orange-brown (Figure 3) and fluoresced orange-pink under ultraviolet light (Figure 4); it was acidic (pH 5.5 to 6.0), and there was proteinuria (100 to 300 mg/100 ml). Tests for bilirubin, occult blood, glucose, and ketones were negative. There were a few fine granular casts and much amorphous vacuolar material, probably lipid droplets, in the urinary sediment.

The results of hematologic examination of Cats B and C are summarized in Table 1. They had severe macrocytic hypochromic anemia [20] and frequently exhibited anisocytosis, poikilocytosis, target cells, and nucleated erythrocytes. Howell-Jolly bodies and erythrocyte refractile bodies were numerous. No blood parasites were observed in any of the samples examined. The second sample from Cat B was taken September 1, 1972, just before the cat was killed. The severe anemia appeared to be a primary cause of the cat's moribund state. This anemia progressed in severity, in spite of continuous therapy with iron and B vitamin supplements, blood transfusions, and parenteral fluids.

Biochemical examination of this serum also revealed severe renal insufficiency. The blood urea nitrogen level was 360 mg, and the creatinine level was 7.4 mg/100 ml of blood.

Table 1—Hematologic Features of Feline Congenital Porphyria

	Range (and mean) of normal values*	Cat B (9/23/71)	Cat B† (9/1/72)	Cat C (1/12/72)
Packed cell volume (%)	24-45 (37)	20	9.3	23
Red cell count $\times 10^6$/cu mm	5-10(7.5)	3.0	1.4	4.35
Hemoglobin (g%)	8-15 (12)	5.1	2.9	7.2
Mean corpuscular volume (cu μ)	39-55 (45)	66.6	66.4	52.9
Mean corpuscular hemoglobin concentration (%)	30-36 (33)	25.5	32.1	32.0
Mean corpuscular hemoglobin (pg)	—	17.0	29.0	31.3
White blood count $\times 10^3$/cu mm	5.5-19.5 (12.5)	14.3	11.0	12.4
Nucleated red cells/cu mm	—	3,900	4,100	1,116

* From Schalm, Veterinary Hematology.[20]
† Sample taken when cat was moribund.

Gross Observations

At necropsy, Cat C was in good flesh, but Cats A and B were thin, and Cat B was dehydrated. In all affected cats, gross and microscopic examination revealed remarkable similarity in lesions. The following description applies to observations in all 3 cats; differences are noted where they occurred.

The bones exhibited dark brown discoloration in ordinary light (Figure 5), which contrasted sharply with their orange-pink fluorescence under ultraviolet light (Figure 6). The marrow was also dark brown.

The thoracic and abdominal viscera were all discolored (in various shades of brown), most severely in liver, spleen and kidneys (Figure 2). Liver and spleen were enlarged, resisted cutting, and bulged at the cut surface. The liver in Cat B was extremely firm and was so discolored that lobular architecture could not be discerned. The gall bladder contained thick, syrupy, brown-black bile.

In the kidneys of Cats B and C, the renal capsule was stripped with difficulty, and the subcapsular surface was slightly pitted. On cut surface the cortex was dark brown and the medulla lighter. The urinary bladder of Cats B and C had 30 to 50 ml of brown urine. The lungs were light tan in Cats B and C. There was about 5 ml of clear tan fluid in the pericardial sac of Cat C.

Microscopic Observations

Two kinds of pigment were visible in tissue sections of most organs. One was present in clumps as well as small granules and was dark brown, isotropic, negative for lipid, and positive for the Prussian blue reaction; it did not fluoresce with ultraviolet light. It was interpreted as hemosiderin.

The other pigment consisted of irregular aggregations of gold-brown material that was isotropic, often contained slight amounts of lipid droplets, and was negative for the Prussian blue reaction. Under ultraviolet light it fluoresced red-orange with various combinations of exciter and barrier filters. The combination yielding the brightest fluorescence of pigment with minimum background fluorescence was the fluorescein isothiocyanate exciter filter (spectral transmission = 350 to 495 nm) with number 50 barrier filter (spectral transmission > 500 nm). This pigment was interpreted as porphyrin. It could be seen best in unfixed frozen sections but was also demonstrable in formalin-fixed paraffin-embedded unstained sections.

Generally, the microscopic tissue changes were less advanced in Cat A (which died following surgery) than in Cats B and C, in which the disease

had progressed to a terminal stage. The kidneys of all cats showed moderate irregular thickening of the basement membranes of the glomeruli with severe thickening of the basement membrane of Bowman's capsule and the convoluted tubules (Figures 7–9). The glomeruli of Cats A and C had slight mesangial hypercellularity, while those of Cat B had severe hypercellularity with moderate proliferation of mesangial matrix. There was reduction of glomerular capillary spaces and occasional adhesions were present between parietal and visceral layers of Bowman's capsule. In Cat C, there was dilation and filling of Bowman's space with proteinaceous material, which was also present in most convoluted tubules. Usually the affected glomerular and tubular basement membrane were located adjacent to one another (Figure 7) and in some sections the thickened basement membrane of Bowman's capsule was observed to be continuous with those of its proximal convoluted tubule. In Cat A, about 20% of the convoluted tubules and Bowman's capsules had basement membrane thickening, while in Cats B and C about 80 to 90% were thickened. The epithelial cells resting upon the thickened basement membrane were attenuated in size, roughly proportionate to the degree of thickening of the basement membrane. Vacuoles of lipid are normally present in the cytoplasm of convoluted tubular epithelium of cats,[21] but they were markedly increased in size and number in Cats B and C. In tubules with greatly thickened basement membrane, no epithelium was visible with the light microscope. Desquamated tubular cells were often seen in the lumen. Proximal convoluted tubules were more severely affected than distal convoluted tubules. Few or none of the basement membrane or epithelial cells in the renal medulla were affected.

The presence of interstitial fibrosis and lymphocytic infiltration was slight in the kidney of Cat A, moderate in Cat B, and severe in Cat C.

Immunofluorescent methods failed to demonstrate the presence of immune complexes in the renal lesions.

In the kidney, porphyrins were the predominant pigment in Cats A and C, occurring primarily in interstitial tissue adjacent to convoluted tubules with thickened basement membrane. Porphyrins were present less often in glomerular tufts and convoluted tubular epithelium. Occasionally some material in the tubular lumens had a brown color that was less intense than that in the interstitial tissues. Hemosiderin was the predominant pigment in Cat B, occurring particularly in glomerular mesangium.

Liver tissue of all cats contained porphyrins in hepatocytes. Hemosiderin was seen in Kupffer cells and, less often, in hepatocytes. In Cat B there was massive accumulation of hemosiderin in centrolobular

Kupffer cells (Figure 10), increase in lipid vacuoles in hepatic cells, and edema of the space of Disse. Connective tissue was slightly increased around central veins.

The spleen showed a decrease in the size of Malpighian corpuscles and an increase in red pulp due to erythrophagocytosis, hemosiderosis, extramedullary hemopoiesis, and prophyrin deposition. Plasma cells and lymphoreticular cells were numerous. Cat B had massive hemosiderosis but no extramedullary hemopoiesis.

There was prominent hemosiderosis of lymph nodes and slight hemosiderosis of bone marrow. There was marked hyperplasia of cells of the erythroid series in bone marrow of Cats B and C. Porphyrin and hemosiderin deposition in histiocytes of bone marrow was prominent in all 3 cats.

Moderate aggregations of hemosiderin and porphyrin were present in histiocytes of lung, and slight amounts were present in heart, pancreas, adrenal, intestine, testicle, and other tissues.

Gross and microscopic features are summarized in Table 2.

Electron Microscopy

Only tissues from Cat B were suitable for electron microscopy. A peculiar membrane-enclosed lamellar body was present intracellularly and extracellularly in kidney and bone marrow (Figures 11–14). It was very pleomorphic and varied in greatest diameter from 150 to 1000 nm,

Table 2—Summary of Gross and Microscopic Features of Feline Congenital Porphyria

	Cat A	Cat B	Cat C
Brown discoloration of teeth, bones, and viscera	+++	+++	+++
Splenomegaly	++	+++	+++
Hepatomegaly	++	+++	+++
Hemosiderosis			
Liver	+	+++	++
Spleen	+	+++	++
Kidney	+	++	++
Bone marrow	+	++	++
Porphyrin deposition			
Liver	+	+	+
Spleen	++	++	++
Kidney	++	++	++
Bone marrow	NE	+++	NE
Kidney			
Glomerular hypercellularity	+	+++	++
Basement membrane thickening	++	++	++
Interstitial fibrosis	+	++	+++
Lipidosis of tubules	+	+++	+++

NE = not examined.

the mean diameter being about 400 nm. The body was bounded by a trilaminar unit membrane and was usually oval in shape, but the membrane often had an irregular, wavy configuration. Most of the membrane-enclosed lamellar body was filled with a homogenous material that had the same electron density as lipid. A constant feature was the presence of an extremely electron-dense lamellar material, resembling myelin figure, usually located eccentrically within the limiting membrane. The dark bands were about 20 to 24 Å thick (mean, 21 Å) and the light bands were about 12 to 17 Å thick (mean, 15 Å). The electron density of the membrane-enclosed lamellar body was present, although diminished, even when sections were unstained with uranyl acetate and lead citrate.

In the kidney, membrane-enclosed lamellar bodies were present in capillary and tubular lumens, Bowman's space, endothelial cytoplasm, between the foot processes of podocytes, within mesangial cell cytoplasm and matrix, and in the interstitial tissue. In the bone marrow, they were most prominent in the cytoplasm of histiocytes and extracellularly.

There was considerable deposition of granular electron-dense material, interpreted as hemosiderin, in mesangial cells of the glomerulus and histiocytes of the interstitial connective tissue. The mesangial cell cytoplasm and matrix were greatly increased and seemed to encroach upon glomerular capillaries (Figure 15). The basement membrane between glomerular endothelium and podocytes was thickened, and foot processes of podocytes were fused in some areas. No electron-dense deposits were observed in the basement membrane, however.

The basement membranes of many proximal convoluted tubules displayed enormous thickening accompanied by severe atrophy and vacuolar degeneration of tubular epithelium (Figure 16). Epithelial cells contained numerous large lipid droplets and clear spaces, and in some tubules there was lysis of cell membranes, releasing cytoplasmic organelles and lipid droplets into the tubular lumens.

Biochemical Studies

Results of biochemical examination are given in Table 3. There were marked increases in uroporphyrin, coproporphyrin, and protoporphyrin in all tissues and body fluids examined from porphyric cats, as compared with controls. The greatest magnitude of increase occurred in uroporphyrin which varied from hundredfold increases in most organs up to almost a ten-thousand-fold increase in urine of Cat B. Porphyrin extract from teeth of this animal contained only uroporphyrin in detectable levels.

Two collections of blood, urine and feces from Cat B, taken almost a

Table 3—Accumulation of Porphyrin in Tissues and Body Fluids From Control and Porphyric Cats

Specimen	Porphyrin*	Control cats (mean values)	Porphyric cats				
			A	B (9/71)	B (9/72)	C (1/72)	C (3/73)
Urine	URO	7.5		70,300	34,900	3,600	
(μg porphyrin/liter)	COPRO	79.0		138		469	
Feces	URO	0.16		1.76	22.2		685
(μg porphyrin	COPRO	2.16		87.0	1,030		45
/g wet weight)	PROTO	4.44		16.6	139		244
Erythrocytes	URO	2.64		440	3,650	215	
(μg porphyrin/dl)	COPRO	3.62		75	41	76	
	PROTO	49.0		666	569	772	
Bone	URO	0.092	207.00		262.00		125.0
(μg porphyrin/g)	COPRO	0.008	1.46		0.21		1.8
	PROTO	0.465	1.79		0.55		0.98
Spleen	URO	0.028	4.84		5.76		62.0
(μg porphyrin/g)	COPRO	0.036	1.78		0.18		2.0
	PROTO	0.260	3.84		0.78		13.3
Liver	URO	0.064	23.2		36.6		15.6
(μg porphyrin/g)	COPRO	0.108	14.0		7.05		1.3
	PROTO	0.182	11.5		0.36		2.4
Kidney	URO	0.098	16.9		14.5		10.7
(μg porphyrin/g)	COPRO	0.030	0.29		0.40		1.2
	PROTO	0.075	3.20		2.7		1.56

* URO = uroporphyrin, COPRO = coproporphyrin, PROTO = protoporphyrin.

year apart (September 1971 and September 1972), revealed about tenfold increases in uroporphyrin in erythrocytes and feces. There was less uroporphyrin in urine, but this may be due to polyuria from the renal disease combined with acidity of the urine. Erythrocytes from Cat B were also analyzed for porphyrin in January 1972, with the results being almost identical with those in September 1971. Thus the marked increase in blood and tissue levels of uroporphyrin occurred between January 1972 and September 1972.

δ -Aminolevulinic acid and prophobilinogen were assayed in urine only from Cat B just prior to euthanasia. In this one specimen, the δ -aminolevulinic acid concentration was 1.8 mg/liter (probably normal) and the porphobilinogen concentration was 16 mg/liter (probably moderately elevated).

The type of porphyrin isomer in the urine of Cats B and C was predominantly Type I, with only traces of Type III present.

Discussion

This feline porphyria is hereditary, but the data are too meager to permit characterization of the type of inheritance. With 2 affected male offspring out of 3 survivors from a mating of affected female and unaffected male siblings, the pattern is consistent with that of either autosomal dominance or recessiveness. There are no affected survivors of the family studied here, but it is unlikely that the genetic defect is lost entirely, and a search is underway in the Seattle area for other affected cats.

The biochemical lesions observed are excessive accumulations of uroporphyrin I, coproporphyrin I, and protoporphyrin in erythrocytes, other tissues, and excreta. The magnitude of increase is generally greatest in uroporphyrin, least in protoporphyrin. The levels of porphyrin present in these cats are much higher than those reported in other cases of feline porphyria.[10-13] Isomer determinations of porphyrins in Cats B and C indicated that the predominant isomer was Type I. This is similar to the congenital erythropoietic porphyrias in man and animals thus far studied.

Studies of human [22] and bovine [23] erythropoietic porphyrias have revealed deficiencies in activity of uroporphyrinogen III cosynthetase in erythrocytes. This is believed to be the principal cause of the increased production of Type I isomers of uroporphyrin and coproporphyrin, which then accumulate in erythrocytes and tissues. Animals are unable to use this porphyrin isomer in the biosynthesis of protoporphyrin and heme. Precise understanding of the defect must await the discovery of other affected cats and subsequent assays of their blood and tissues for activity of the various enzymes associated with heme biosynthesis.

In the feline porphyria described by Glenn and his colleagues [10-13] no photosensitivity, renal disease, or deposition of porphyrins in viscera is reported. Anemia is not severe enough to affect the general health of the cats. In the porphyria described herein, there appear to be three interrelated disease processes: a) photosensitivity, b) hemolytic anemia, and c) renal disease. There is no experimental evidence that photosensitivity was present in the porphyric cats. However, all 3 cats had episodes of nasal and ocular discharge during the summer. Since they were all kept indoors most of the time and since intense sunlight is prevalent in this area only during the summer months, the history of nasal and ocular discharge occurring during these months in all 3 cats is suggestive of photosensitivity.

The hypochromic macrocytic anemia and the hemosiderosis and extramedullary hematopoiesis are indicative of hemolytic anemia, which could well be a response to the deranged heme biosynthetic pathway.

Studies of bovine porphyria have revealed an inverse correlation between erythrocytic porphyrin concentration and erythrocyte half-life.[24] The accumulation of porphyrins in erythrocytes is believed to result in hemolysis with decreased erythrocyte half-life.[25] The mechanism of this hemolysis remains obscure, however. The severe anemia thus appears to be related to the extremely high concentrations of porphyrins in erythrocytes.

Deposition of porphyrins in teeth and bone is believed to be due to their affinity for mineral components.[3] The accumulation of prophyrins in soft tissues in this porphyria is possibly the result of phagocytosis of porphyrin-containing erythrocytes by resident reticuloendothelial cells in the organs involved. Another result of this phagocytosis of erythrocytes would be hemosiderosis, which was quite severe in Cats B and C.

Severe renal disease and renal failure, such as were present in these cats, have not been reported in other porphyrias. Since the glomerular lesion was characterized by proliferation of mesangial cells and matrix, accumulation of much hemosiderin in the mesangium, and narrowing of glomerular capillaries, it is probable that the tubular injury is due to chronic ischemia. The tendency for adjacent tubules and glomeruli to be involved suggests that the disease has affected the entire nephron, beginning with glomerular mesangium. It may be that phagocytosis of porphyrin-containing erythrocytes or their by-products by the mesangial cells, active components in the reticuloendothelial system, leads to the mesangial proliferation. This then results in glomerular capillary narrowing and chronic ischemic injury to the tubule.

Since extremely high levels of uroporphyrin were excreted in the urine, one may also postulate that the presence of persistently high concentrations of this or other hemoglobin-related compounds may have injured tubular and glomerular epithelial cells and the mesangium, resulting in degeneration, necrosis, and replacement of tubular epithelium, with excessive synthesis of basement membrane materials. If true, it would seem likely that this would have occurred in severe cases of some of the other porphyrias.

Alternatively, an entirely separate renal disease, possibly also with an hereditary component, may have been present in these cats. Cram *et al.*[26] have described and reviewed a total of 17 cases of human porphyria coexisting with systemic lupus erythematosus. However, in these cats we were unable to demonstrate deposits of immunoglobulin in kidney tissue, a feature of the immune complex glomerulonephritis seen in systemic lupus erythematosus.

The peculiar membrane-enclosed lamellar bodies observed in kidney and bone marrow of Cat B by electron microscopy appear to contain

phospholipid and other lipid materials and are probably abnormal lysosome-like structures that have been released from damaged cells. It is possible that the production and release of such bodies is hereditary and is related to the same aberrant genetic information that led to the development of the porphyria. Alternatively, such bodies may be the result of nonspecific degenerative changes in cells as a result of the severe anemia, hypoxia, and uremia associated with this porphyria. The membrane-enclosed lamellar bodies bear some similarity to the extracellular phospholipid bodies observed in human cases of renal disease.[27] They are also somewhat similar to the vacuoles that appear to contain lipid, which have been observed in the basement membrane in cases of human hereditary nephritis.[28]

In summary, a comparison of this congenital erythropoietic porphyria with that previously described in cats [10-13] indicates that, although there are many similarities, the porphyria described here has two characteristic features: a) the levels of porphyrins are much higher in the erythrocytes and viscera; and b) there is severe renal disease and anemia associated with the porphyria.

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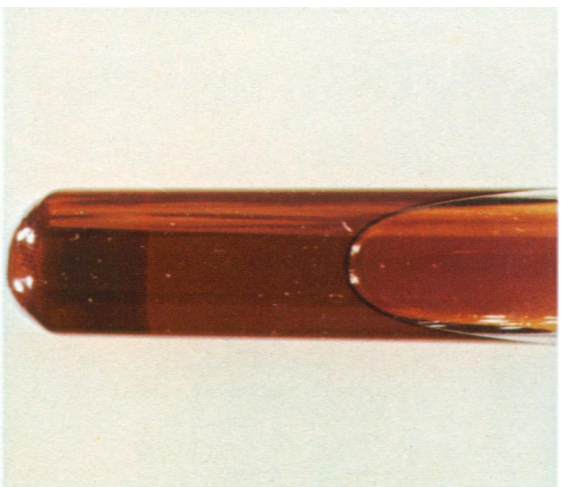

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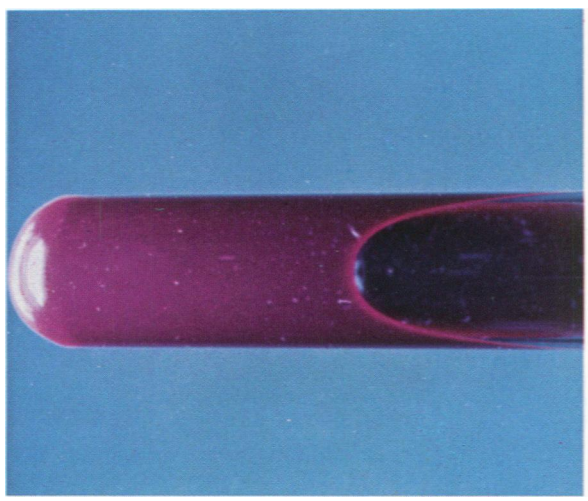

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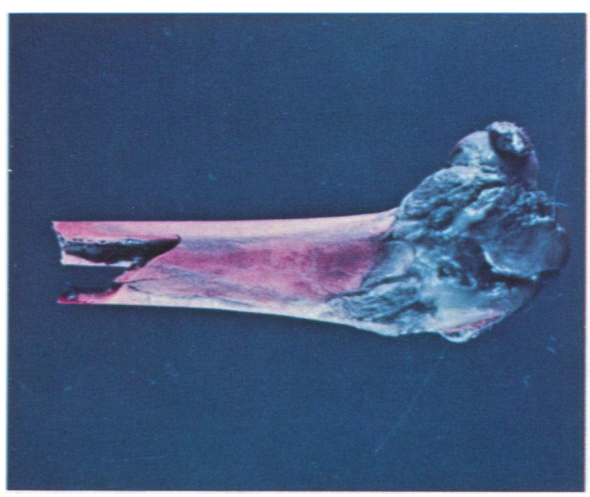


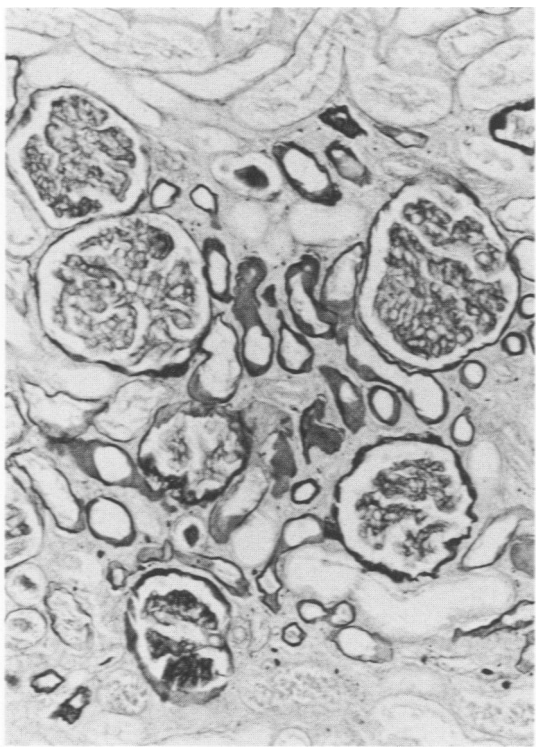
Figure 1—Orange-brown discoloration of teeth in Cat A. **Figure 2**—Orange-brown discoloration of kidney in Cat A; discoloration is more intense in subcapsular portion of cortex and decreased in papillus. **Figure 3**—Orange-brown color of urine in Cat B. **Figure 4**—Orange-pink fluorescence of the same urine under ultraviolet light. **Figure 5**—Dark brown discoloration of femur in Cat A. **Figure 6**—Orange-pink fluorescence of the same femur under ultraviolet light.

Figure 7—This and all subsequent figures are from Cat B. There is loss of renal tubules, atrophy of tubular epithelium and thickening of basement membrane of Bowman's capsule and adjacent convoluted tubules. Other tubules are unaffected. (PAS, × 160)

Figure 8—In this higher magnification of kidney, there is mesangial hypercellularity, thickening of basement membranes of Bowman's capsule and adjacent convoluted tubules, tubular atrophy, and interstitial fibrosis and infiltration of lymphocytes (H&E, ×400).

Figure 9—Proliferation of mesangial cells and matrix, deposition of granular pigment (hemosiderin) in mesangium, tubular atrophy, basement membrane thickening, and interstitial fibrosis are evident in this section of kidney (Toluidine blue, × 900).

Figure 10—In this section of liver there is fibrosis around the central vein, with hemosiderin in Kupffer cells and fatty metamorphosis of hepatocytes (H&E, × 400).



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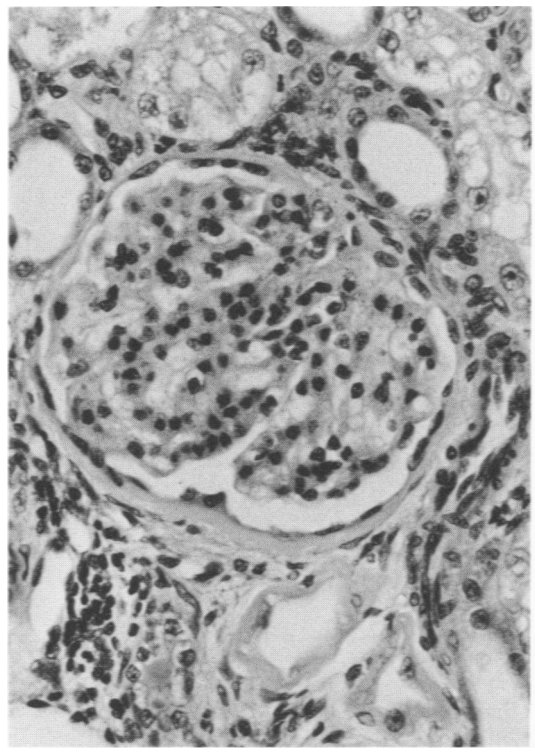

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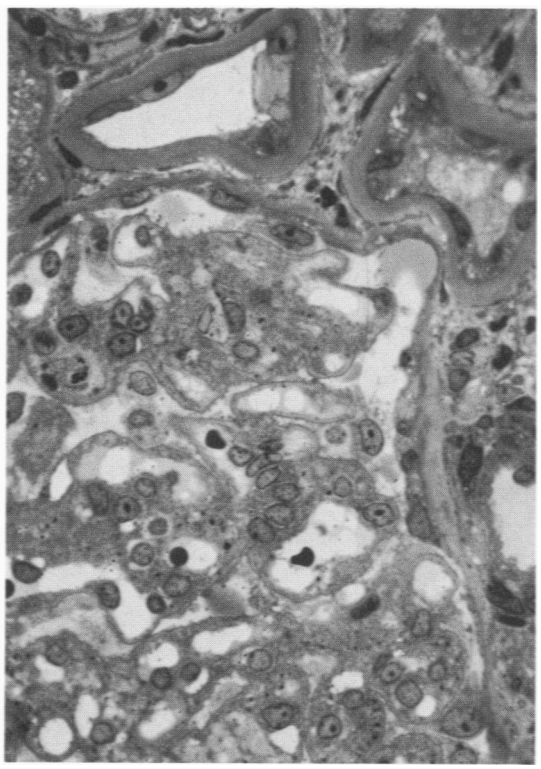

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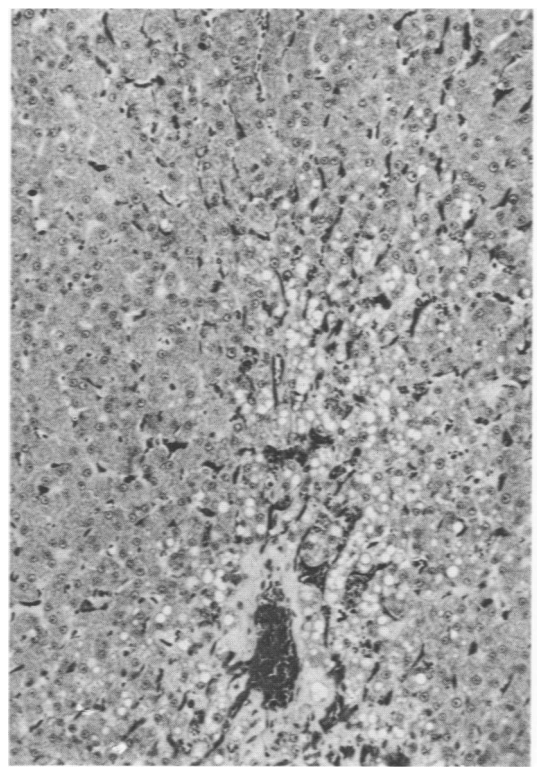

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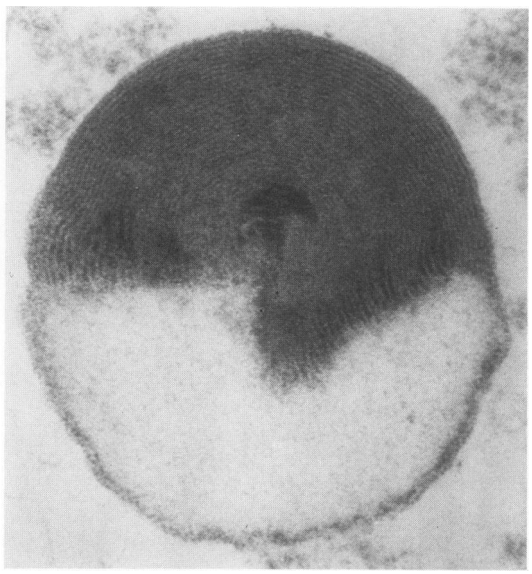

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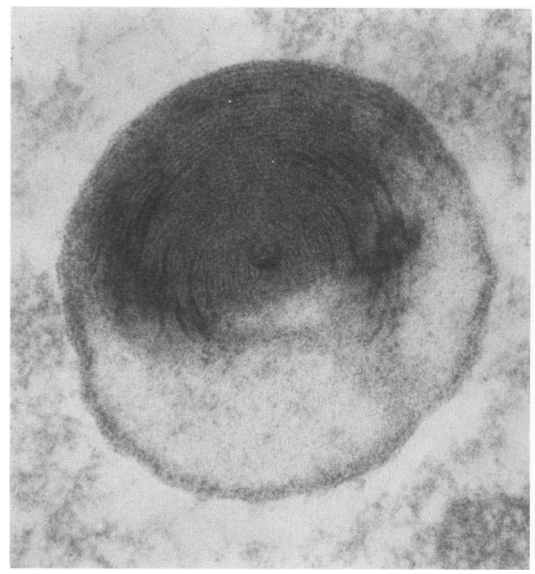

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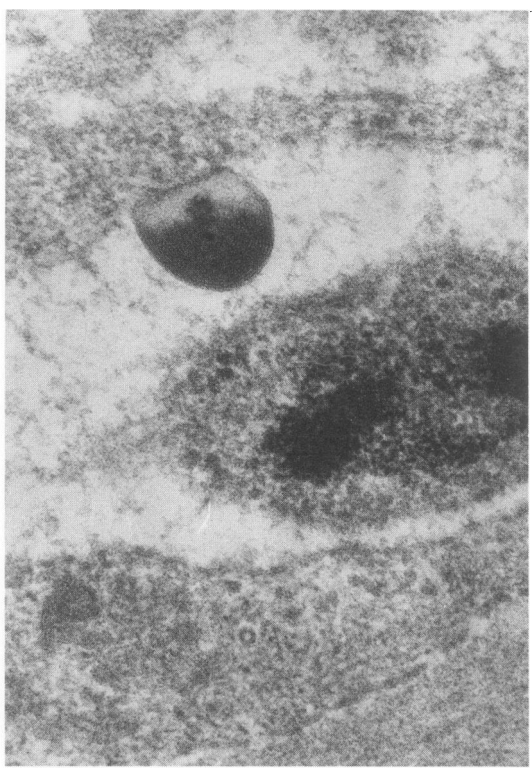

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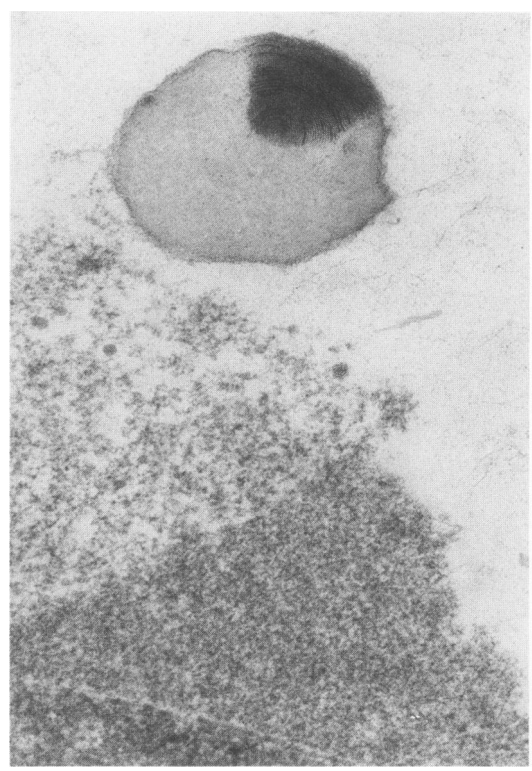

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Figure 11—Membrane-enclosed lamellar body (MELB) in Bowman's space of kidney (Uranyl acetate and lead citrate, × 150,000). **Figure 12**—MELB in filtration space between foot processes of podocytes (Uranyl acetate and lead citrate, × 150,000). **Figure 13**—MELB within filtration slit of podocyte in glomerulus (Uranyl acetate and lead citrate, × 90,000). **Figure 14**—MELB adjacent to erythrocyte in bone marrow (Uranyl acetate and lead citrate, × 60,000).

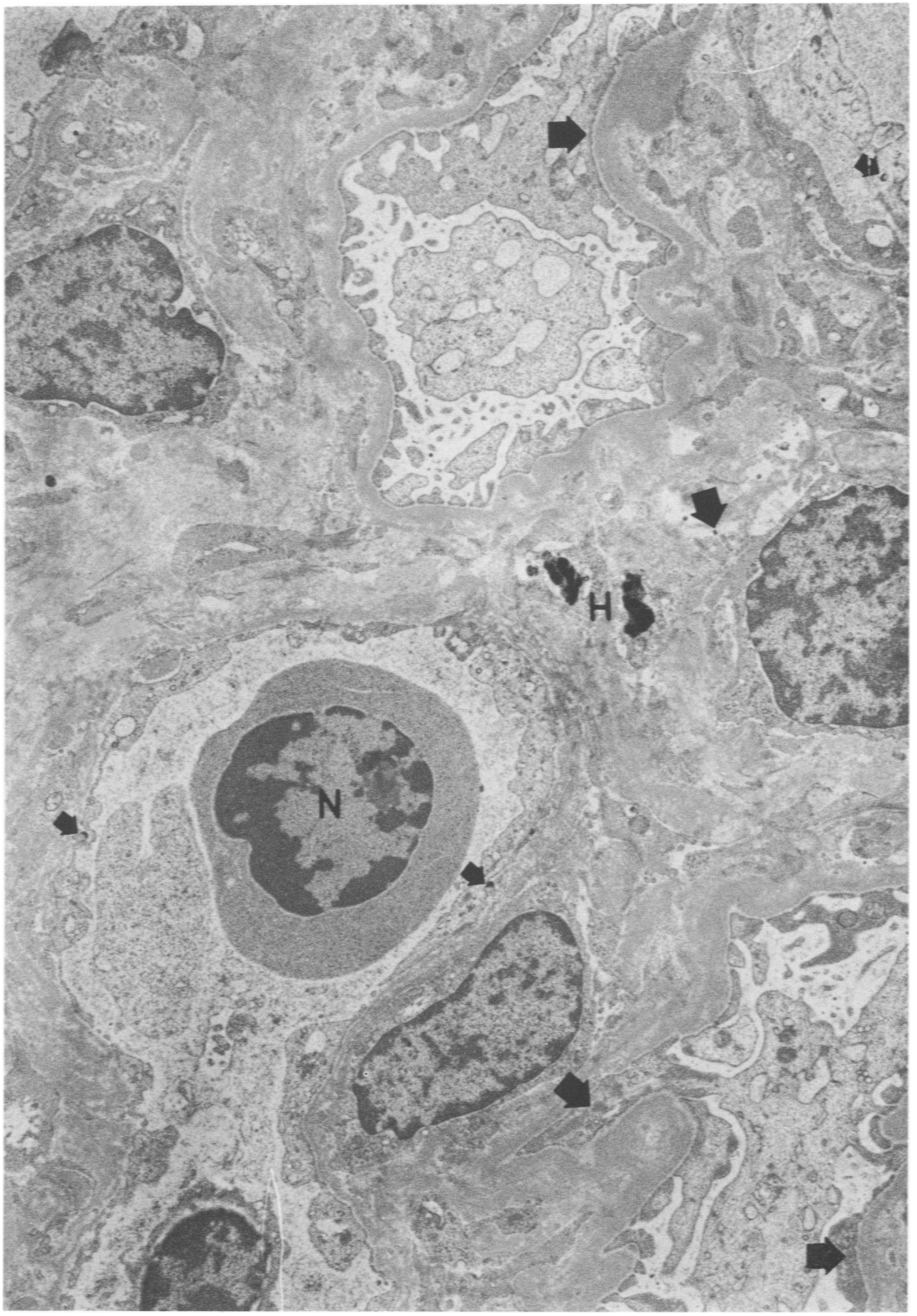

Figure 15—In this glomerulus there is extensive proliferation and thickening of mesangial cells and mesangial and epithelial basement membranes with accumulation of hemosiderin and hemoglobin by-products (*H*). A nucleated erythrocyte (*N*) is present in a glomerular capillary. There is some fusion of foot processes of the podocytes (*large arrows*). MELBs are present throughout the tissue (*small arrows*). (× 7500)

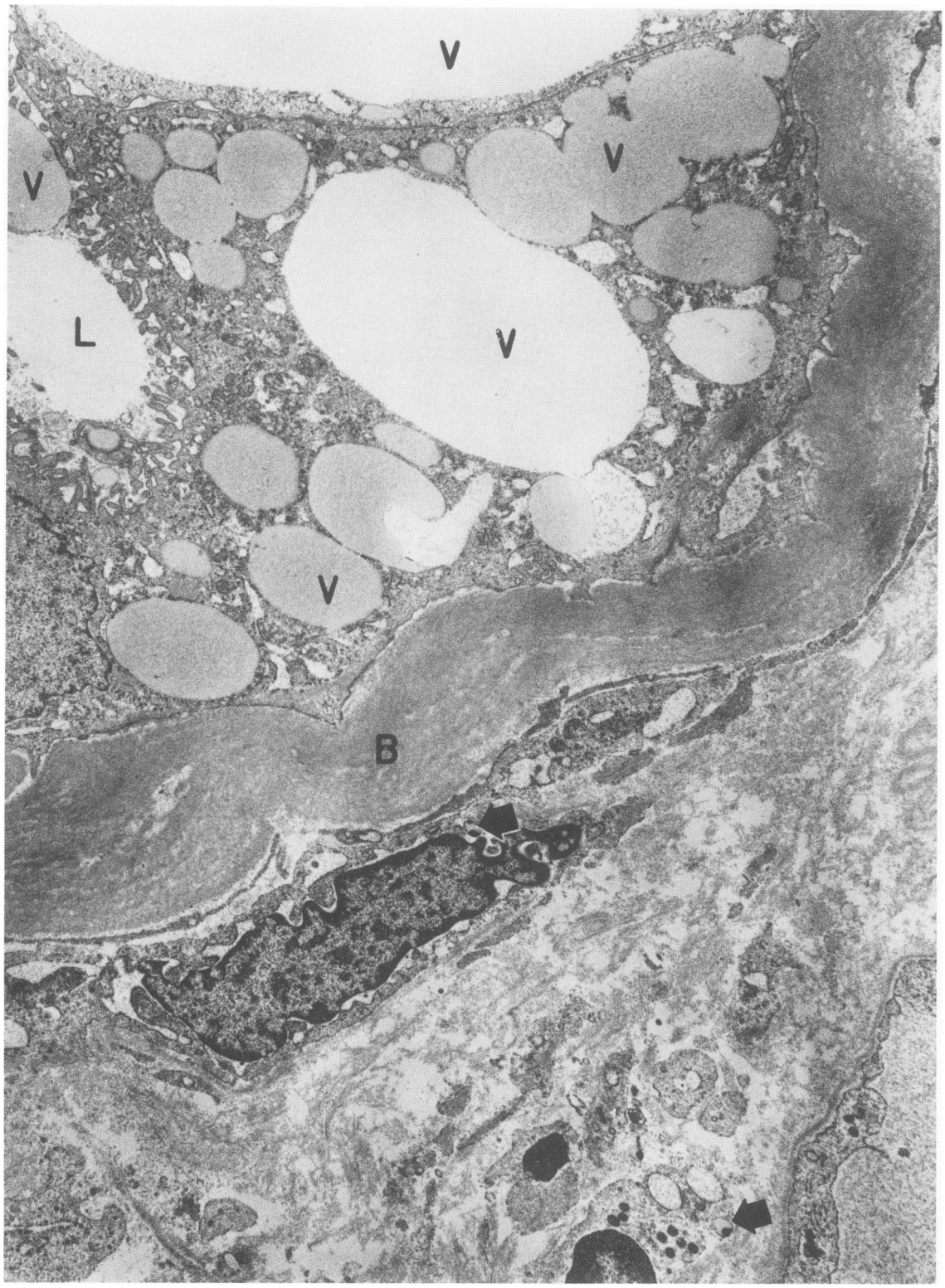

Figure 16—Convoluted tubule of kidney. There is atrophy of the tubular epithelium and numerous empty and lipid-filled vacuoles (*V*) are present in the cytoplasm and in the tubular lumen (*L*). The basement membrane (*B*) is enormously thickened in a laminar pattern. Fibroblasts and collagen are increased in the interstitium. Several MELBs are present in the cytoplasm of interstitial cells (*arrows*). (× 8100)