Spontaneous amyloidosis in twelve chimpanzees, *Pan troglodytes*

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Abstract: Spontaneous amyloidosis was diagnosed in 11 male and 1 female chimpanzees and confirmed histologically and immunohisto-chemically. The chimpanzees were ≥ 15 years of age when first diag-nosed and averaged 22.4 years of age. The average survival time after diagnosis of systemic amyloidosis was 1.86 years with a standard devia-tion of 4.06 years (n = 7). The chimpanzees with amyloidosis were asymptomatic except for hepatomegaly, which became more detectable with age. Significant increases in clinical chemistry values, as compared with referenced normals and established normals, of blood urea nitro-gen (BUN), asparate aminotransferase (AST), gamma-glutamyltrans-ferase (GGT), globulin, total protein, creatinine phosphokinase (CPK), sedimentation rate, and triglycerides were found in animals 7 years of age or older with amyloidosis. These serum chemistry values, while increased in chimpanzees with amyloidosis, were generally within nor-mal limits. Immunohistochemistry for both amyloid A protein and amyloid P component-labeled extracellular amyloid in all chimpanzees with amyloidosis was determined. Amyloid was deposited primarily in the liver. Amyloidosis in the chimpanzee is a chronic, intractable, pro-gressive, fatal disease, and appears to be similar to secondary amy-loidosis in other species.

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Introduction

Amyloidosis is a general term for a group of dis-eases characterized by the excessive deposition of ultrastructurally identical but biochemically dis-tinct protein fibrils either focally or multifocally in body tissue. Amyloid may be deposited in many locations but is most frequently found in spleen, liver, kidney, and adrenal. It is generally a chronic, progressive, insidious disease of unknown cause, which may not become clinically apparent until major organ dysfunction occurs because of dis-placement, atrophy, or death of normal cells [1–4].

Amyloid can be seen grossly if the deposits are large and replace tissue to an extent that the tissue appears pale tan to white, is firm, and the organ is enlarged or distorted in shape. A gross diagnosis is possible by applying an iodine solution to the affected site and rinsing it with a weak solution of sulfuric acid. If the material is positive for amyloid it will turn yellow with iodine and blue-violet with sulfuric acid [8]. Histologically, amyloid stained with hematoxylin and eosin (H&E) appears as eosinophilic hyalinized material, which can be globular or linear and may be minimally fibrillar. To distinguish it from collagen, other proteins, or fibrin, it may be stained for light microscopic eval-uation with Congo red, Thioflavin T or S, Tolu-idine blue, and for immunohistochemistry using amyloid-specific antisera. Congo red stains amy-loid orange to red with normal light and apple green with polarized light, Thioflavin T or S causes fluorescence of amyloid, and Toluidine blue causes a red polarization of amyloid [2].

Amyloid is generally classified as primary or idiopathic when there is no associated disease; sec-ondary or reactive, which is associated with chronic infectious or inflammatory disease; or fa-milial, which is unassociated with other diseases but is characterized by distinctive types of

neuropathy, cardiopathy, and nephropathy [3]. Primary amyloidosis is associated with plasma cell dyscrasias and is the most common type in humans but is rare in animals. Secondary or reactive amyloidosis is characterized by tissue amyloid A protein and is associated with chronic inflammatory disease and is the most common form in animals [2–4, 18, 30]. Serum amyloid A protein is produced by the liver and released into the blood during acute generalized infections, cancers, and familial diseases. Serum amyloid A in the blood is converted to AA and deposited in tissue [4]. About 95% of amyloid is fibril protein and 5% is the P component, which is a glycoprotein. The two most common amyloid proteins are the amyloid light-chain and the amyloid A protein. The amyloid P component is distinct from amyloid fibrils but closely associated with all forms of amyloid [8].

Spontaneous amyloidosis in nonhuman primates has been reported in squirrel monkeys (*Saimiri sciureus*), rhesus (*Macaca mulatta*), baboons (*Papio hamadryas*), drill baboons (*Mandrillus leucophaeus*), cynomolgus (*Macaca fascicularis*), Formosan rock macaques (*Macaca cyclopis*), pig-tailed macaques (*Macaca nemestrina*), Celebes macaques (*Macaca nigra*), Diana monkeys (*Cercopithecus diana*), tree shrews (*Urogale everetti*), *Microcebus murinus*, orangutans (*Pongo pygmaeus*), and chimpanzees (*Pan troglodytes*) [4–6, 10–12, 14, 16, 21–23, 26, 27, 29, 30]. Amyloidosis is best documented in rhesus, pigtailed macaques, cynomolgus macaques, baboons, and squirrel monkeys. To the best of our knowledge, there are only three peer-reviewed publications reporting amyloidosis in a total of four chimpanzees. One case reported amyloid in the brains of two aged female chimpanzees whose health status and tissue at other sites was not mentioned [11]. The second case reported was that of Ham, the space chimpanzee, who died at 26 years of age with hepatic amyloidosis [16]. Amyloid was not found in any other tissues and Ham apparently did not have any other chronic disease condition. The third case was a chimpanzee death due to amyloid A amyloidosis. Amyloid was recognized in the liver, heart, spleen, kidney, and testicle [23].

The purpose of this paper is to inform people about the existence, diagnosis, and clinical course of amyloidosis in the chimpanzee.

Materials and methods
Animals

The 11 adult males and 1 female adult chimpanzees were maintained at two facilities. The housing was similar at both facilities and consisted of metal and concrete indoor–outdoor cages. Animals were fed commercial monkey diets supplemented with a variety of other foods and water was available *ad libitum*. Initial diagnosis in all cases was via liver biopsy either because the animal was on a research project that required liver biopsies, or because it had an enlarged liver.

Clinical features

Lifetime records of clinical histories, disease status, and research use were available for each ape. The records were reviewed for abnormal clinical signs, research use, and disease status.

Clinical pathology

Clinical chemistry data were evaluated in the chimpanzees for possible value in diagnosing amyloidosis without a liver biopsy. The clinical chemistries were taken from routine clinical pathology data in the chimpanzee's lifetime records. Albumin, alkaline phosphatase, alanine aminotransferase (ALT), asparate aminotransferase (AST), total bilirubin, blood urea nitrogen (BUN), cholesterol, creatinine phosphokinase (CPK), creatinine, gamma-glutamyltransferase (GGT), globulin, lactate dehydrogenase (LDH), sodium, total protein, and triglycerides were evaluated. These chemistries were chosen primarily because they reflect the health of the liver and kidney and because adequate lifetime data were available to evaluate each of them [13, 28]. Sedimentation rates were also evaluated for chimpanzees with amyloidosis only and compared with values available in a published reference [13].

Pathology

Complete necropsies were performed and complete sets of tissues taken from the seven apes that died or were killed. All tissues, including the liver biopsies, were fixed in neutral buffered 10% formalin, processed conventionally, embedded in paraffin, cut in 5-μm sections, and stained with H&E. Congo red staining and immunohistochemistry were done on all tissues suspected of containing amyloid when viewed by light microscopy.

Immunohistochemistry

Immunohistochemistry was performed using a monoclonal mouse antibody that recognizes human amyloid A protein and a polyclonal rabbit antibody that recognizes human P component (DAKO Corporation, Carpinteria, CA). An

munoperoxidase method (Envision System; DAKO Corporation [9]) was used to detect both antigens. Immunolabeling for P component was also done using a labeled streptavidin-biotin (LSAB) procedure. The antibody for amyloid A protein was diluted 1:500 and the antibody for P component was diluted 1:100. Tissue sections were deparaffinized and rehydrated before immunohistochemistry. Sections immunolabeled for amyloid A protein were pretreated in a solution of 0.01% trypsin (Sigma Chemical, St Louis, MO) in phosphate-buffered saline (PBS) for 30 minutes before application of the primary antibody. Sections labeled for P component received no pretreatment. The immunoperoxidase procedure was performed according to the manufacturer's instructions [9]. Prior to application of the primary antibody, sections labeled by the LSAB procedure were incubated with normal goat serum for 20 minutes.

Fig. 1. Significant differences in clinical chemistry reference values, established normal chimpanzee values, and chimpanzees 7 years old or older with amyloidosis.

Following the primary antibody incubation, the LSAB procedure was completed by sequential application of biotinylated goat anti-rabbit secondary antibody (Vector Labs, Burlingame, CA) diluted 1:200 for 30 minutes, alkaline phosphatase-labeled streptavidin reagent (Bethesda Research Laboratories, Gaithersburg, MD) for 30 minutes, and Histomark Red (Kirkegaard and Perry Laboratories, Gaithersburg, MD) chromogenic substrate for 50 minutes in the dark. All incubations in both procedures were performed at room temperature. Duplicate sections of each tissue were incubated with normal mouse serum or normal rabbit serum as negative controls. Following immunohistochemistry, all tissue sections were counterstained with Gill's hematoxylin.

Statistics

Thirty-seven randomly selected clinically normal colony chimpanzees, which had not been used on experiments and had complete lifetime records with at least ten data points for each clinical chemistry test evaluated, were selected as controls. Since the number of clinical chemistry tests were not numerous and did not generally correspond to the chimpanzees with amyloidosis clinical chemistry test time points, the chimpanzees were grouped into four age groups: 0–3, 4–6, 7–10, and > 10 years of age. There were inadequate data available for chimpanzees less than 7 years of age so these data could not be properly evaluated. The means were established for each normal chimpanzee and for each chimpanzee with amyloidosis from all available data points from lifetime records for each clinical chemistry test. Next, the means were established for each age group from all the individual means in that age group. The normal means were compared with referenced means [13] and with means for the chimpanzees with amyloidosis using the nonparametric method of the Kruskal–Wallis test [7]. Since there were incomplete data for three of the chimpanzees with amyloidosis and two had hepatitis viral infection, only 7 of 12 chimpanzees with amyloidosis data were compared with the established normal and referenced data (Table 2, Fig. 1). Sedimentation rates were only compared with published data [13].

Results

Clinical features

Table 1 is an overview of the 12 cases of chimpanzees with amyloidosis. The only clinical sign was hepatomegaly.
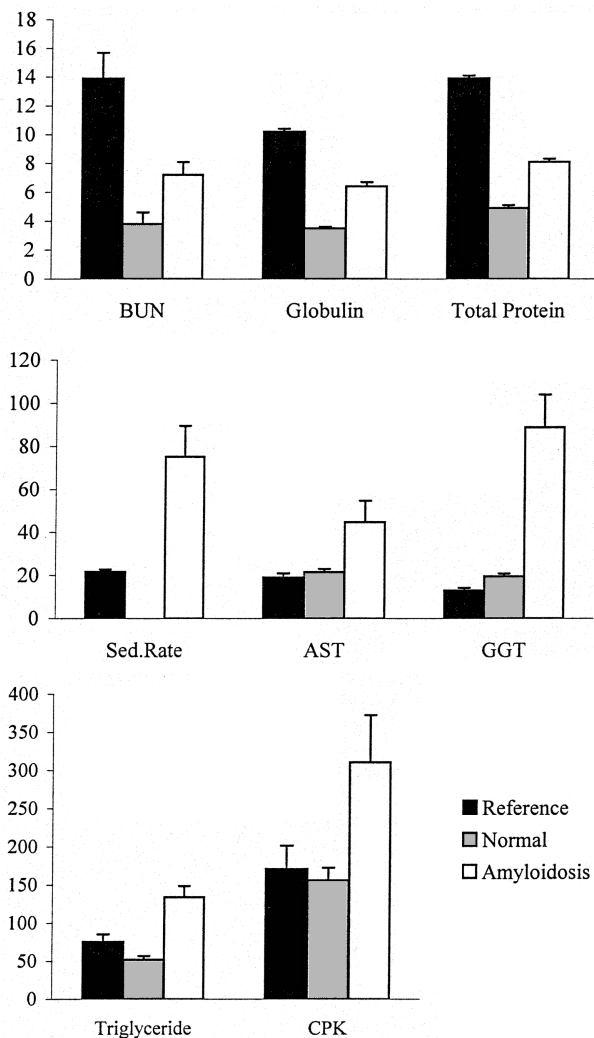
Table 1. Spontaneous amyloidosis in 12 chimpanzees (*Pan troglodytes*)

Animal ID	Sex	Age (yr)		Amyloid					Tissue positive for amyloid							Chronic disease	Clinical signs
		Diagnosis	Death	H&E¹/CR	A	P	EC	CA	Liver	Adr	Spl	Skin	Kid	Lung	Pancreas		
Ken 4X0221	M	19	30	+	+	+	+	–	+	+	–	–	+	–	–	HBV+	Hepatomegaly
Sam 1 4X0213	M	28	28	+	ND	ND	NA	NA	+	+	–	+	–	–	–	Unknown	Hepatomegaly
Flint 4X0392	M	15	16	+	+	+	+	–	+	+	+	–	–	+	+	Cellulitis	Hepatomegaly
Rock 1730	M	32	32	+	+	+	+	–	+	–	+	+	–	–	–	Nephritis	Hepatomegaly
Grinch 4342	M	16	17	+	+	+	+	–	+	–	*	–	–	–	–	Malaria	Hepatomegaly
Willie H-3751	M	23	23	+	–	–	–	–	+	–	+	–	–	–	–	Unknown	Hepatomegaly
Neptune H-4256	M	20	20	+	–	–	–	–	+	–	–	–	–	–	–	Unknown	Hepatomegaly
Len 4X0009	M	19	NA	+	+	+	–	+	+	NA	NA	NA	NA	NA	NA	HCV+	Hepatomegaly
Plato 3670 4X0148	M	18	NA	+	+	+	+	+	+	NA	NA	NA	NA	NA	NA	Unknown	Hepatomegaly
Barash 3570	M	18	NA	+	+	+	+	–	+	NA	NA	NA	NA	NA	NA	Unknown	Hepatomegaly
Polly 1756	F	31	NA	+	ND	ND	NA	NA	+	NA	NA	NA	NA	NA	NA	Unknown	Hepatomegaly
CJ 5915	M	30	NA	+	ND	ND	NA	NA	+	NA	NA	NA	NA	NA	NA	Unknown	Hepatomegaly

¹Abbreviations used in table: H&E, hematoxylin and eosin; CR, Congo red; EC, extracellular; CA, cell associated; Adr, adrenals; Spl, spleen; Kid, kidney. ND, not done; NA, not applicable.
*Splenectomized for malaria study.

Clinical pathology

There were no significant differences in albumin, alkaline phosphatase, ALT, cholesterol, creatinine, total bilirubin, LDH, sodium, and chloride between normal and amyloid cases. Significant increases, while generally within normal limits, were found in BUN, AST, GGT, globulin, total protein, sedimentation rates, and triglycerides within the 7 years and older group (Table 2, Fig. 1). The only variation in these results was that triglycerides were not significantly increased in chimpanzees with amyloidosis in the 7–10 year group when compared with established normals. CPK was only elevated when compared with referenced normal values [13]. The sedimentation rates were significantly increased in 7 years and older chimpanzees with amyloidosis as compared with referenced values [13].

Pathology

The apes that died had markedly enlarged, pale tan to yellow livers (Fig. 2). Amyloid deposits were not recognized in other tissues during gross examination.

All of the amyloid-associated lesions had a common histological appearance, including deposition of an eosinophilic, hyalinized, fibrillar material around vessels and in extracellular locations (Figs 3 and 4). This material stained orange to red with the Congo red stain and was birefringent with polarized light. It stained well with immunohistochemical stains for both amyloid A and P (Figs 5 and 6). Amyloid deposition was generally the most severe and obvious in the liver. The deposits began in the space of Disse between Kupffer's cells and hepatocytes and were seen along hepatic cords with extension into sinusoidal spaces. These amyloid deposits were associated with atrophy and necrosis of hepatocytes. Central veins and portal areas were spared except in severe, advanced cases. In general, the amyloid deposition in other tissues was minimal and not easily recognized. In the spleen, amyloid was seen primarily in lymphoid follicles in germinal centers around central arterioles. In the adrenals, amyloid was usually found at the cortical-medullary junction and associated with vessels in the zona fasciculata. In the kidney it was found in glomerular tufts primarily in the mesangium and basement membranes. Subcutaneous amyloid, while found in only one chimpanzee, was difficult to discern because of inflammation and necrosis, but it was associated with collagenous connective tissue and vessels after Congo red staining. Amyloid deposition in the

Table 2. Significant differences in clinical chemistry reference values, established normal chimpanzee values, and chimpanzees with amyloidosis values by age[1]

	Published clinical chemistry reference values[2]			Age groups (yr)	Normal chimpanzees				Chimpanzees with amyloidosis[3]				Chi-squared (df = 1)	P-value[5]
	Mean	SE	Range		n[4]	Mean	SE	Range	n[4]	Mean	SE	Range		
Sed. rate (mm/h)	21.7	1.04	0–53	7–10	–	–	–	–	2	6.63	2.88	3.8–9.5	–	–
				>10	–	–	–	–	7	78.66	14.56	4.0–124	–	–
				≥7	–	–	–	–	7	75.20	37.89	8.7–124	3.736+	0.0359[5]
AST (μ/l)	19.0	2.0	9–29	7–10	22	17.95	2.56	12.0–22.6	4	33.26	5.54	24.0–48.0	9.849	0.0017[5]
				>10	37	23.80	13.16	12.0–90.0	7	47.50	12.17	14.9–99.3	5.875	0.0154[5]
				≥7	59	21.62	1.41	12.0–90.0	7	44.81	9.93	14.9–86.5	7.509	0.0061[5]
BUN (mg/dl)	13.9	1.77	4–22	7–10	22	9.85	1.90	7.0–15.4	4	13.39	0.77	11.4–14.8	6.964	0.0083[5]
				>10	37	10.47	7.52	6.0–53.7	7	13.53	1.03	10.3–19.2	12.059	0.0005[5]
				≥7	59	10.24	0.79	6.0–53.7	7	13.91	0.90	12.2–19.2	14.841	0.0001[5]
GGT (μ/l)	13.0	1.2	7–19	7–10	18	17.95	5.12	9.0–29.0	1	16.33	–	–	–	–
				>10	33	20.31	11.12	9.1–65.0	5	94.11	15.04	58–147	12.427	0.0004[5]
				≥7	51	19.48	1.32	9.0–65.0	5	88.91	15.17	58–147	13.248	0.0003[5]
Globulin (g/dl)	3.8	0.20	2.8–4.8	7–10	22	3.31	0.78	0.4–4.0	4	3.50	0.08	3.3–3.7	0.005	0.9433
				>10	37	3.55	0.97	0.3–5.7	7	5.10	0.26	4.6–6.5	14.966	<0.0001[5]
				≥7	59	3.46	0.12	0.3–5.7	7	4.87	0.28	4.0–6.2	14.779	0.0001[5]
Total protein (g/dl)	7.2	0.16	6.4–8.0	7–10	22	5.88	2.21	0.7–7.8	4	7.51	0.12	7.2–7.8	5.351	0.0207[5]
				>10	37	6.68	1.61	0.7–8.5	7	8.19	0.17	7.5–8.8	13.771	0.0002[5]
				≥7	59	6.38	0.24	0.7–8.5	7	8.10	0.15	7.5–8.7	16.279	<0.0001[5]
Triglyceride (mg/dl)	76.0	9.61	27–125	7–10	9	58.13	19.41	38.6–100	3	84.77	12.69	69.8–110	3.769	0.0522
				>10	5	41.87	10.63	29.9–56.7	7	150.66	13.72	104.0–196	8.077	0.0045[5]
				≥7	14	52.32	4.87	29.9–100	7	134.22	14.57	83.4–196	12.824	0.0003[5]
CPK (μ/l)	171.0	30.79	14–328	7–10	9	130.90	12.45	76–193	1	762.33	–	–	–	–
				>10	5	202.46	31.68	125–298	7	298.37	54.06	71.1–473	2.380	0.1229
				≥7	14	156.46	10.23	178–317	7	310.76	61.63	71.1–560	4.681	0.0305[5]

[1]Except for sed. rate all statistical results are established normal data vs. data from chimpanzee with amyloidosis.
[2]The comparison of reference values vs. chimpanzees with amyloidosis values is by t-test.
[3]Does not include chimpanzees infected with hepatitis virus.
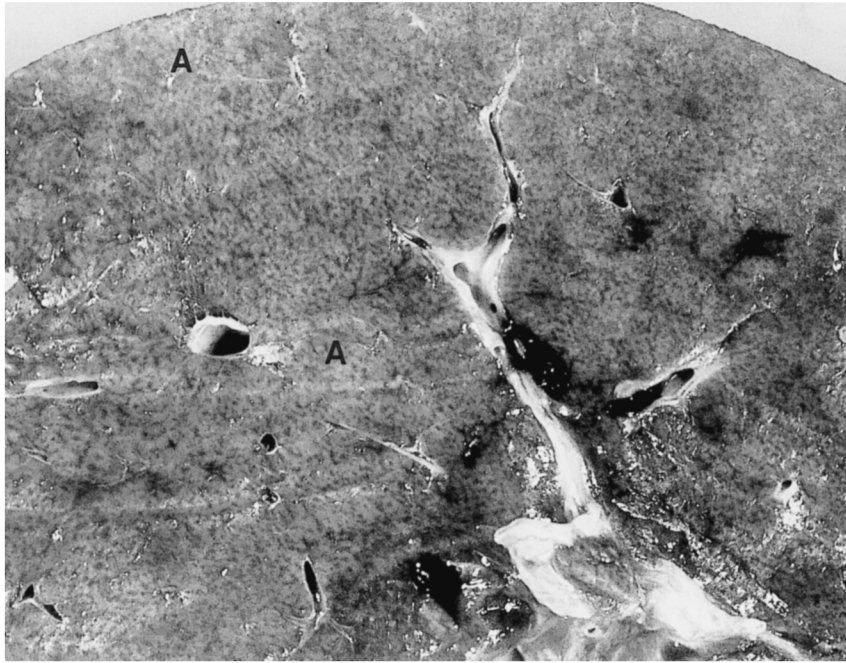[4]n = number of chimpanzees.
[5]P-value <0.05 is significant.

Fig. 2. Cut section of a liver with marked amyloid deposition. Note the light tan areas with a blending into the darker areas of more normal liver.

lung was most obvious in the alveolar capillaries. Pancreatic amyloid was deposited around interstitial vessels. If the amyloid deposits were massive, it was not possible to establish their initial deposition sites. It was not seen in any other tissues in these chimpanzees, including the stomach and intestines.

Statistics

No significant differences were found in albumin, alkaline phosphatase, ALT, total bilirubin, chloride, cholesterol, sodium, creatinine, or LDH levels. Significant increases were found in BUN, AST, GGT, globulin, total protein, sedimentation rates, and triglycerides in 7 years and older chimpanzees with amyloidosis (Fig. 1). The power calculations indicate that the comparisons of total protein, globulin, GGT, and triglyceride between control and abnormal chimpanzees have 99% power to detect the mean difference with a significance level of $P = 0.05$. However, there is only 42% power for BUN. All statistical analyses were performed with the Statistical Analysis System (SAS) [25]. All power analyses were performed by the Solar Power Analysis (SPA) [24].

The average age of diagnosis of amyloidosis was 22.4 years with a standard deviation (SD) of 6.2, and the average survival time after diagnosis was 1.86 years with a SD of 4.06 (n = 7). One chimpanzee lived 11 years after diagnosis.

Discussion

Light-chain primary amyloidosis is rare in animals and is primarily a disease of humans with plasma cell dyscrasias [8]. Evidence of such plasma cell dyscrasia was not seen in any of the chimpanzees in this study. The mouse antibody to human amyloid A protein we used has been shown not to react with antigens such as human serum proteins (albumin, transferrin, IgG) and non-AA amyloid fibril proteins [9, 19, 20], thus ruling out light-chain-associated amyloid in the deposits present in the chimpanzee tissues. The amyloid P component immunohistochemistry is less specific, but served to confirm the tissue localization of amyloid A immunoreactive material.

The liver appears to be the primary site of amyloid deposition in these chimpanzees, because all cases had amyloidosis of the liver but not necessarily in other locations. It is known that serum protein amyloid is produced in the liver and stimulated by interleukin 1 produced by mononuclear phagocytes. It is not known why the serum amyloid is deposited in some patients and not in others, or why the specific tissue is susceptible to deposition [30]. In other species with amyloid the spleen or kidney is the most common site for amyloid deposition [3, 8, 17]. In the rhesus, amyloid is preferentially deposited in the spleen, liver, kidneys, intestine, and mesenteric lymph nodes, which is similar to the human, baboon, and
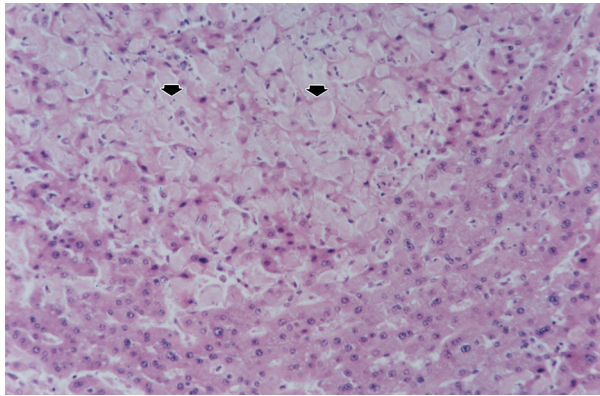
Fig. 3. This section of liver has areas of focal heavy deposition of amyloid surrounding areas of light deposition of amyloid, and normal liver (H&E, 100 ×).
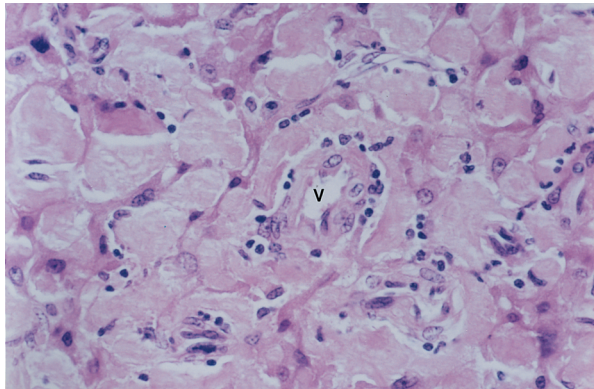

Fig. 4. This is a higher magnification of heavy amyloid deposition in the liver. Note the deposition in vessel wall and in intracellular and extracellular locations. Most hepatocytes are atrophic or lost (H&E, 200 ×).
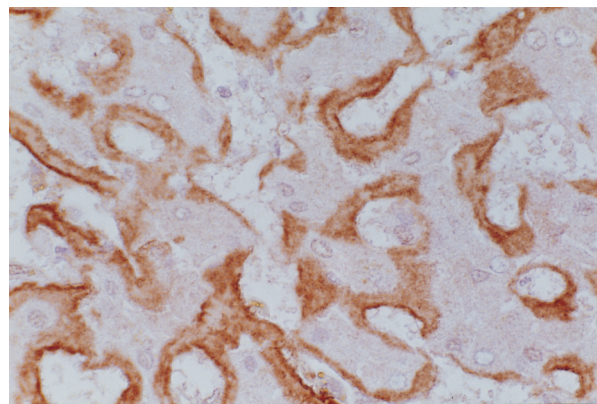

Fig. 5. Immunohistochemical stain for amyloid P in the liver of a chimpanzee using monoclonal rabbit anti-human amyloid P component antibodies showing amyloid deposition in extracellular locations (immunoperoxidase procedure, Gill's hematoxylin and counterstain, 60 ×).
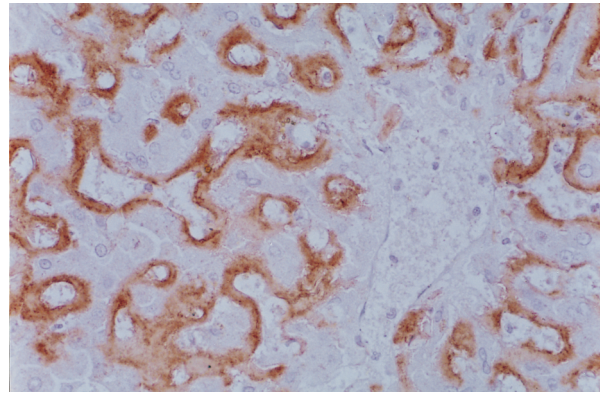

Fig. 6. Immunohistochemical stain for amyloid A in the liver of a chimpanzee using monoclonal mouse anti-human amyloid A component antibodies showing amyloid deposition in extracellular locations (immunoperoxidase procedure, Gill's hematoxylin and counterstain, 40 ×).

pigtailed macaques reactive amyloidosis [30]. There is no obvious reason for this phenomenon.

A preponderance of the cases were diagnosed in male chimpanzees even though the two colonies contained approximately equal numbers of females and males. Again no obvious reason is known for the phenomenon but genetic influences and aging effects should be considered [30].

Amyloidosis was diagnosed at a relatively young age in the 12 chimpanzees we studied and does not seem to necessarily be associated with chronic disease as currently hypothesized [3, 8, 17, 18, 29, 30]. Of course, it is the rare chimpanzee that does not have chronic low-grade intestinal parasitisms [15, 26]. It is apparent that clinical pathology values are only an indication that the chimpanzee may have amyloidosis, since the values, while elevated, are still generally within normal limits. A liver biopsy is still the only diagnostic test that can give a positive diagnosis [8]. In our experience, the liver is the biopsy organ of choice because it is the one organ that consistently has amyloid deposition in chimpanzees with amyloidosis.

Therefore, a clinician with a hepatomegalic chimpanzee 7 years of age or older with high normal or elevated clinical chemistries listed in this paper should consider amyloidosis in the disease differential. After the diagnosis of amyloidosis is made the prognosis is poor.

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