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The clinical and pathologic features of a 7-yr-old Macaca mulatta that died of generalized amyloidosis were reported. Prior to death, marked changes in serum proteins, characterized by decreased levels of albumin and an increase in globulins were observed. At necropsy, dense deposits of amyloid were present in the liver, spleen, adrenal glands, and gastrointestinal tract. Lesser amounts of amyloid were present in the kidney, lymph nodes, thyroids, and bone marrow. The animal also had a chronic arthritis of the femoral-tibial joints. The monkey had received whole-body radiation 5 yr prior to death, but its influence on the subsequent development of amyloidosis was unknown.

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GENERALIZED AMYLOIDOSIS IN A RHESUS MONKEY[1,2,3,4,5]

HAROLD W. CASEY,[6] JOHN H. KIRK, AND GARY A. SPLITTER

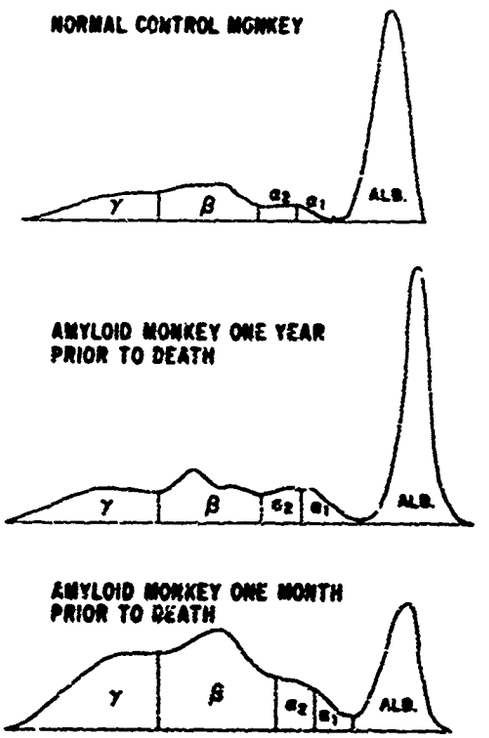
SUMMARY • *The clinical and pathologic features of a 7-yr-old Macaca mulatta that died of generalized amyloidosis were reported. Prior to death, marked changes in serum proteins, characterized by decreased levels of albumin and an increase in globulins, were observed. At necropsy, dense deposits of amyloid were present in the liver, spleen, adrenal glands, and gastrointestinal tract. Lesser amounts of amyloid were present in the kidney, lymph nodes, thyroids, and bone marrow. The animal also had a chronic arthritis of the femoral-tibial joints. The monkey had received whole-body radiation 5 yr prior to death, but its influence on the subsequent development of amyloidosis was unknown.*

The following report describes the clinical and pathologic features of a male 7-yr-old rhesus monkey (*Macaca mulatta*) with generalized amyloidosis and chronic arthritis of the femoral-tibial joints. The monkey was from a colony of approximately 400 animals of the same species that are under life-time observation for long-term radiation effects. Death occurred 62 mo after 600 rads of whole-body radiation with 55 Mev protons. Specific radiation effects of 55 Mev protons on monkeys from the same group have previously been reported for up to 5 yr post-irradiation (1, 2).

Case Report

Clinical findings: After the monkey recov-

Fig 1. *Serum electrophoretic pattern of normal monkey and amyloid monkey at 1 yr and at 1 mo prior to death. Note marked decrease in albumin and concurrent rise in globulin fractions in the amyloid monkey.*



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[3] The animals involved in this study were maintained in accordance with the "Guide for Laboratory Animal Facilities and Care" as published by the National Academy of Sciences—National Research Council.
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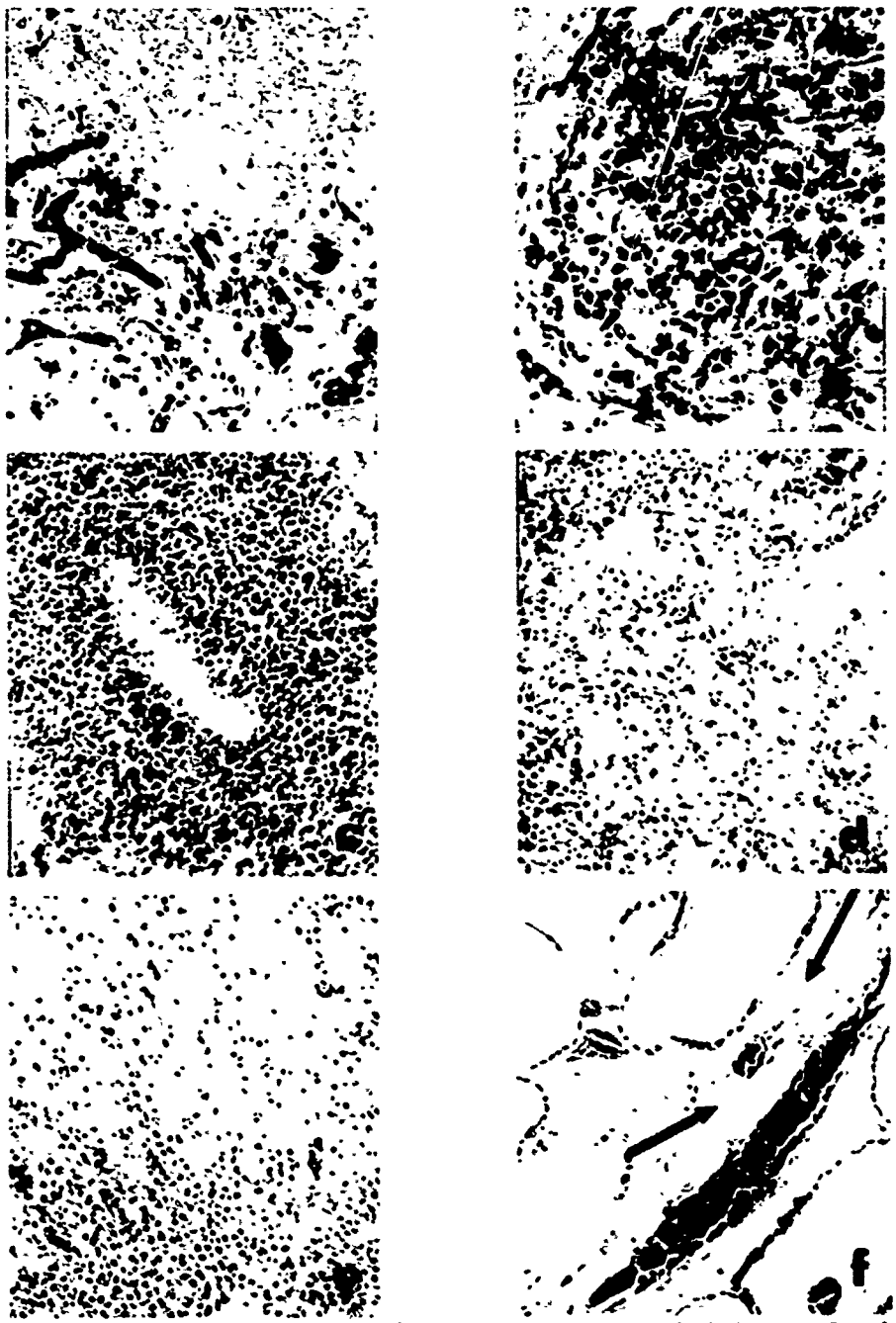


Fig 2. *H&E stained sections. a) Section of liver with extensive amyloid deposits. Portal areas containing bile ducts remain visible but hepatic cords have been replaced with amyloid. 150 X. b) Section of liver with hepatic cords remaining but with amyloid deposits in space of Disse. 150 X. c) Amyloid deposits in splenic follicles. Amyloid in follicular area was located in and adjacent to walls of central arteries. 150 X. d) Red pulp of spleen, illustrating heavy amyloid deposits that have obliterated numerous sinusoids. 150 X. e) Adrenal gland at cortico-medullary junction with intensive amyloid deposits radiating upward along sinusoids of zona reticularis. 150 X. f) Amyloid deposits in interstitium of thyroid gland. 150 X.*

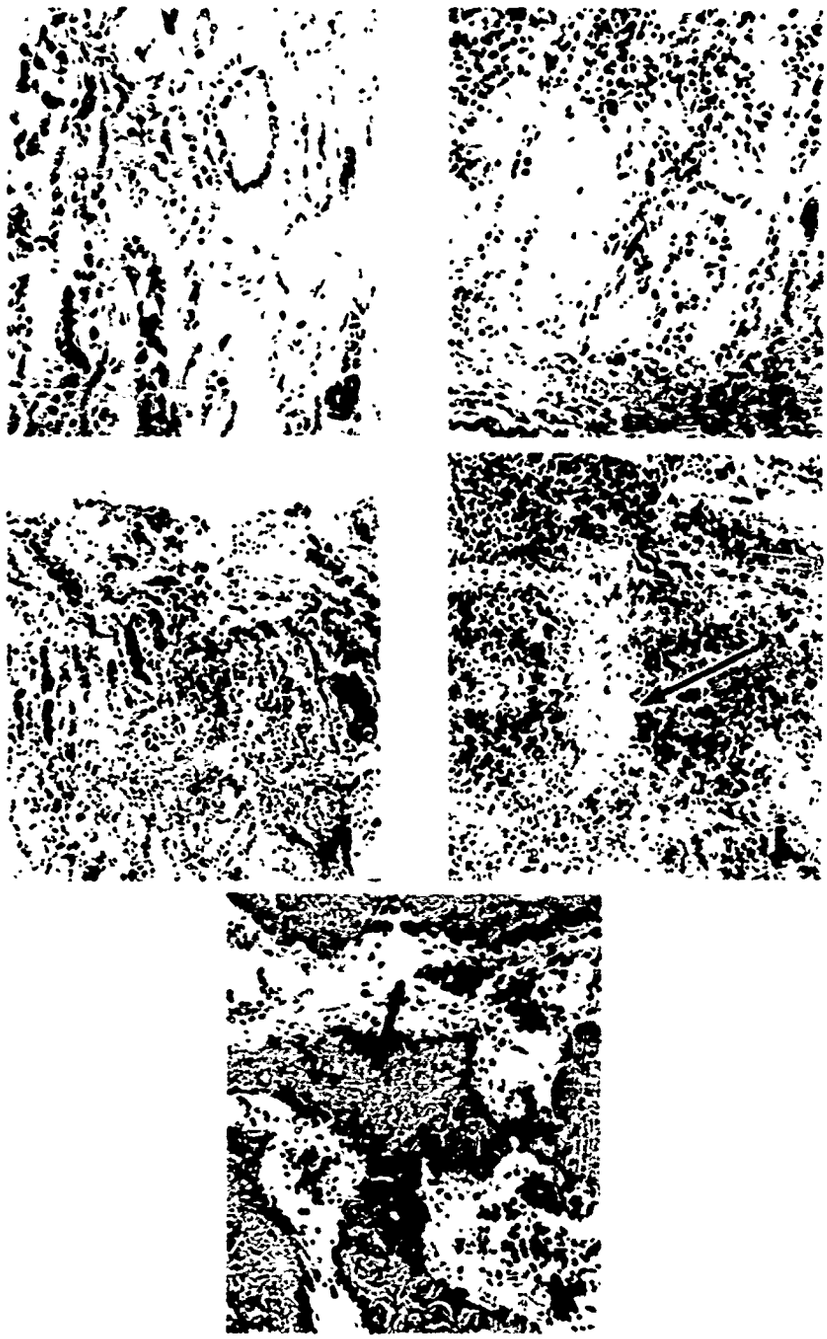


Fig 2. *(cont) H&E stained sections. g) Kidney medulla with heavy interstitial amyloid deposits. Tubule cells have some autolytic sloughing. Glomeruli contained only faintly distinguishable amyloid. 150 X. h) Mucosa of stomach. Heavy amyloid deposits have destroyed and distorted gastric glands. 150 X. i) Section of duodenum with extensive amyloid in lamina propria that extends into submucosa around Brunner's glands. 66 X. j) Lymph node with light deposit of amyloid around lymphoid follicle and adjacent cortical sinus, arrow. 150 X. k) Section of thoracic vertebra with light deposits of amyloid (arrow) adjacent to trabeculae. 150 X.*

ered from the acute hematologic effects of the radiation, the first indication of disease was detected approximately 3½ yr prior to death when an elevated serum alkaline phosphatase level, 20.4 Sigma units, was observed. Elevated alkaline phosphatase levels continued until death, fluctuating between 23 and 40. Isozyme studies were not performed on the serum alkaline phosphatase. Serum glutamic oxalate transaminase was never above normal. Marked changes in plasma proteins, with an inversion of the A/G ratio, were evident approximately 12 mo before death (Fig 1). Just prior to death, albumin levels decreased to 1.9 g/100 ml compared to 4.22 g/100 ml in normal animals.[7] Concomitantly, plasma globulins increased to 6.4 g/100 ml versus the 3.5 g/100 ml for normal monkeys.[7] Most of the globulin increases were in the alpha and gamma globulins. Total leukocyte and platelet numbers showed no specific alterations after the animal recovered from the hematologic radiation effects approximately 6 mo post-exposure. Two mo prior to death, hemoglobin values dropped to 9.3 g/100 ml with a packed cell volume of 32. Just prior to death, these values decreased to 8.0 and 28, respectively. No significant changes were observed in blood glucose, urea nitrogen, or creatinine levels. Urinalyses were not performed. Approximately 2 mo before death, the animal became reluctant to move, and clinical examination revealed limited mobility of both knee joints. The animal died at night and was necropsied the following morning.

Pathology: At necropsy the animal was in a poor nutritional condition. Externally, no lesions were present; however, the legs were fixed in a partially flexed position at the femoral-tibial joint, and the mass of posterior leg muscles was reduced. On dissection, the femoral-tibial joint cavities were difficult to distinguish, as extensive firm adhesions filled the entire cavity and completely covered or obliterated articular cartilages. No gross lesions were present in any other joints of the appendages. Gross lesions in the thoracic cavity were limited to numerous focal granulomas that contained lung mites, *Pneumonyssus simicola*. Abdominal lesions consisted of a greatly enlarged liver, 700 g (normal, approximately 125 g). All lobes of the liver were uniformly enlarged, very friable, and tan to light orange. On cross section the orange-tan tissue was dissected by irregular areas of dark red tissue. The spleen was normal sized and greyish; on cross-section, follicles were not distinguishable. Other than irregular congestion and some evidence of postmortem change, remarkable changes were not seen in other abdominal organs.

Histologically, essentially all organs were characterized by extensive amyloid deposits that were birefringent in sections stained with Congo Red and fluoresced with UV light in sections stained with thioflavine T.

Amyloid deposits in the liver had essentially destroyed the normal architecture. In most areas, only isolated hepatocytes and bile ducts remained embedded in the amyloid (Fig 2a). Even though the hepatic cords and sinusoids were obliterated, irregular-shaped nuclei, interpreted as Kupffer cells, remained embedded in the amyloid. In an estimated 10% of the tissue examined, hepatic lobules were distinguishable (Fig 2b); however, in these areas amyloid was visible in the space of Disse. Hepatocytes that remained were generally atrophic, and their cytoplasm contained small vacuoles.

Although extensively infiltrated with amyloid, the splenic architecture was still distinguishable. The follicles were atrophic and contained small numbers of lymphocytes; however, active germinal centers were not present. Amyloid deposits in follicles were limited to small areas around some central arteries (Fig 2c). In contrast, the sinusoidal areas contained dense amyloid deposits (Fig 2d). The deposits were situated in what were considered interstitial areas that had compressed or obliterated many sinusoidal channels.

[7] Mean for 8 specific control monkeys of the same age maintained in the same colony.

In the adrenal glands, amyloid deposits were generally limited to areas adjacent to the cortico-medullary junction, primarily along the sinuses of the zona reticularis that occasionally dissected in the zona fasciiata and the medullae (Fig 2e). Both thyroids had large follicles filled with dense colloid and the follicular cells were squamous-like in appearance. Amyloid deposits were seen in some perifollicular capillary areas (Fig 2f). In the kidney, amyloid was found extensively in the medullary interstitium, resulting in distorted kidney tubules (Fig 2g). Amyloid was only faintly distinguishable in mesangial areas of the glomeruli with amyloid stains.

In the digestive tract, amyloid was present in the stomach, duodenum, jejunum, and ileum but was not demonstrable in the large intestine, although multiple sections were examined. Unfortunately, the tongue was not examined histologically. The lamina propria (Fig 2h) of the stomach had irregular deposits of amyloid that in some areas extended from the muscularis mucosa to the luminal epithelial cells. In the duodenum, extensive amyloid was clearly distinguishable in the lamina propria, the villi, and crypts (Fig 2i). Heavy deposits were also present beneath the basement membrane of the Brunner's glands. Amyloid was deposited in the lamina propria of the jejunum and ileum; but the central lacteals of the villi remained patent. Amyloid deposits were not as heavy in the distal portions of the small intestine as in the duodenum.

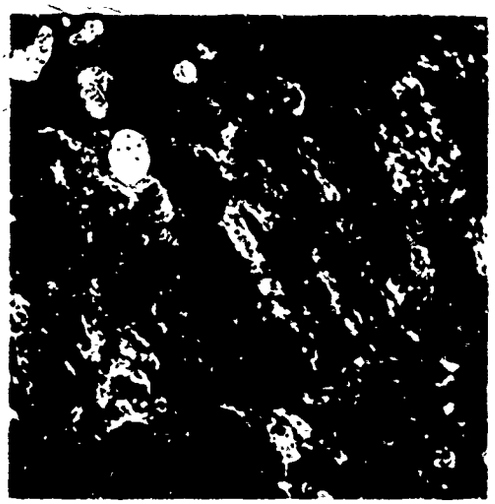
Lymph nodes were moderately atrophic, and active germinal centers were not present. Light deposits of amyloid were present in both the cortex (Fig 2j) and medulla adjacent to the capillary walls, and occasionally in germinal centers. Marked erythrophagocytosis was seen in the sinuses of some nodes.

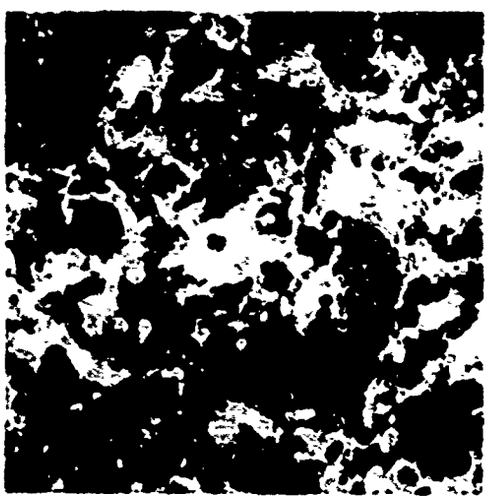
Bone marrow sections from the sternum and thoracic vertebrae had mild hypoplasia, as only approximately 60% of the marrow was occupied by cellular elements. Even though the hemoglobin levels were below normal, active erythropoiesis was present. Amyloid was seen in focal areas of the marrow adjacent to the boney trabeculae (Fig 2k).

The histologic appearance of amyloid deposits in the duodenum as visualized with polarized light in Congo Red stained sections is illustrated (Fig 3a). Amyloid deposits in

Fig 3. a) ***Birefringence (polarized light) of Congo Red stained amyloid in interstitium of villi and Brunner's glands of duodenum. 145 X.***

b) ***Thioflavin T strained section of splenic red pulp illustrating fluorescence with ultraviolet light. BG 12 exciter filter, 470μ barrier filter. 380 X.***





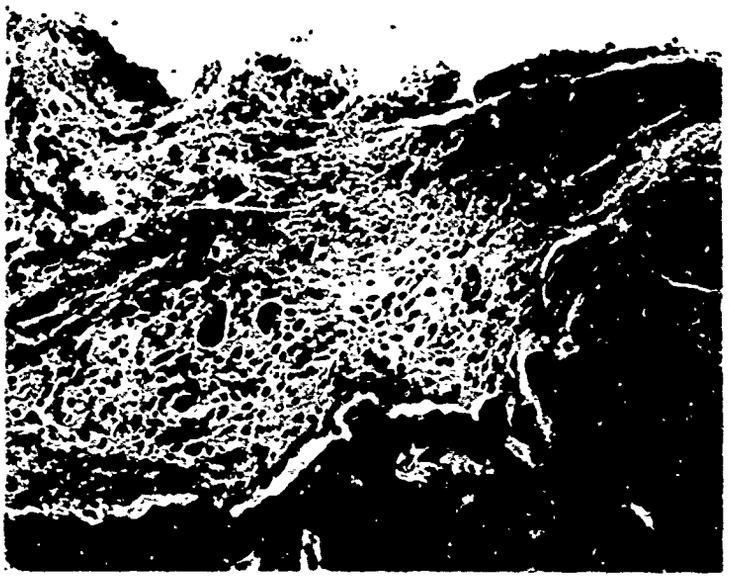


Fig 4. Section of the distal femoral articular cartilage. Note erosion, necrosis, and fibrous tissue proliferation over the articular surface. H&E stain. 150 X.

the splenic red pulp stained with thioflavin T and photographed with ultraviolet light are shown (Fig 3b).

Amyloid was not seen in lung sections, although the basement membrane of the major bronchi showed homologous eosinophilic thickening in some areas. Amyloid was not detected in the heart, salivary glands, pancreas, genital organs.

Histologically the femoral-tibial joints had severe destruction of the articular cartilage characterized by fibrosis and necrosis (Fig 4). The fibrous tissue was more extensive adjacent to the joint capsule but extended across the entire articular surface and dissected into the remaining articular cartilage. Villous formations of fibro-synovial tissue were present in some areas. Small numbers of lymphocytes and plasma cells were present in some areas of the fibrous tissue in addition to synovial cells.

Discussion

Amyloidosis has been previously reported in nonhuman primates. Banks and Bullock (3) described amyloidosis in a squirrel monkey with active chronic suppurative inflammation of the thoracic vertebrae. In this instance the amyloidosis was classified as secondary, and deposits were seen in the spleen, kidney, liver, adrenals, and lymph nodes. The most significant clinical laboratory finding in the squirrel monkey was an elevated BUN, probably attributable to amyloid deposits in the kidney. Alterations in serum proteins were not mentioned. Gillman and Gilbert (4) reported 4 cases of amyloidosis in baboons. All 4 cases were classified as primary, and necropsy data were presented on 3 animals with extensive amyloid deposits of most body organs. Of special interest, 1 baboon also had extensive arthritis of the rheumatoid type, and it is well established that in man amyloidosis is frequently associated with rheumatoid arthritis (5). Clinical features of the amyloidosis in baboons were not extensively tabulated. As the diagnosis of amyloidosis in the present case was made at necropsy, studies were not done that definitely established whether the arthritis was or was not of the rheumatoid arthritic type.

The effects of the previous radiation exposure on the development of the case of amyloidosis described in this report is un-

known. In this respect amyloidosis is not considered a specific late effect of irradiation. In one study of mice it was demonstrated that the final incidence of amyloidosis was the same in irradiated and non-irradiated mice; however, the irradiated mice developed the disease at a younger age (6). No additional cases of amyloidosis have been diagnosed in the colony, but liver biopsies were performed on 5 additional monkeys. The 5 monkeys were chosen for study after a review of the clinical laboratory records of all colony monkeys. All 5 animals had normal serum protein fractions, 1 had an elevated serum alkaline phosphatase level, and 4 had slightly elevated SGOT values. Amyloid was not present in any of the biopsy specimens, although a mild non-specific hepatitis was seen in 3 of the liver specimens.

In the case presented herein, the marked alteration of the serum proteins was probably a partial reflection of the severe liver damage that resulted in decreased albumin production. The reason for the elevated serum globulins is less clear, although hyperglobulinemia is frequently seen in latter stages of both naturally occurring and experimental amyloidosis (7). Eitzman (8) has reported an increase in gamma globulin levels with increasing age in the rhesus monkey; however, in the present case the affected monkey's serum levels clearly exceeded the levels of the control monkeys maintained in the colony.

The elevated alkaline phosphatase levels with decreased serum albumin levels suggest liver disease; however, since isozyme studies were not done, a possibility exists that the elevated phosphatase levels were due to the arthritic lesions. The actual diagnosis of amyloidosis in the case described herein was made at necropsy. A liver biopsy would have permitted an accurate antemortem diagnosis; however, prolonged bleeding may be encountered with biopsies in cases with severe alterations of serum proteins.

In keeping with the current views (7,9), the present case of amyloidosis is not classified as primary or secondary. As the etiology in both categories is not well understood, the present case tends to emphasize the difficulties of classification into distinct type. Although in the case reported herein there was some tissue destruction and inflammation associated with chronic arthritic lesions, it was not extensive enough to clearly warrant classification as secondary amyloidosis.

REFERENCES

1. Lindsay JR, Dalrymple GV, Ghidoni JJ, *et al*: Some effects of 55 MEV protons on primates. *Radiat Res* 28: 446–464, 1966
2. Traynor JE, Casey HW: Five year follow-up of primates exposed to 55 MEV protons. *Radiat Res* 47: 143–148, 1971
3. Banks KL, Bullock BC: Naturally occurring secondary amyloidosis of a squirrel monkey (*Saimiri sciureus*). *J Am Vet Med Assoc* 151: 839–842, 1967
4. Gillman J, Gilbert C: Primary amyloidosis in the baboon (*Papio ursinius*). *Acta Med Scand* 152: 155–189, 1955
5. Ennevaara K, Oka M: Rheumatoid arthritis with amyloidosis. *Ann Rheum Dis* 23: 131–138, 1964
6. Lesher S, Gralin D, Sallese A: Amyloidosis in mice exposed to daily gamma irradiation. *J Natl Cancer Inst* 19: 1119–1127, 1957
7. Cohen AS: Amyloidosis. *N Engl J Med* 277: 522–530, 1967
8. Eitzman DV: Immunoglobulin levels in the *Macaca mulatta*. *Folia primatol* 12: 313–316, 1970
9. Azar HA: Amyloidosis. In *Pathology Annual*, Sommers SC, Ed, New York, Appleton-Century-Crofts, 1968, pp 105–122